

How to pay less for crude petroleum

WHAT is to be done about the price of petroleum? In particular, how is it to be reduced? These are unspoken questions in the backs of many minds, especially in countries (industrialised and developing) that are largely dependent on imported oil. The questions are so often unspoken because they seem dangerous questions, an impression that can only be strengthened by the continued presence of an American fleet in the Persian Gulf (even if for an unconnected purpose). The result, however, is that oil consumers have, since the meeting of the Organization of Petroleum Exporting Countries (OPEC) in Kuwait in October 1973, become catatonically accustomed to the notion that the price of oil will be a monotonically increasing function of time. This grand indifference cannot much longer persist. Something will have to be done about the price of oil. And it goes without saying that military expedients of any kind can form no part of an acceptable strategy by which the oil consumers could interrupt the apparently inexorable upward trend of prices.

The need for a break in the present pattern is now self-evident. The economic consequences of the tenfold increase of the price of oil (in real terms) since 1973 are now everywhere to be seen. Although it would be wrong to blame the increased price of oil for all the economic ills of either industrialised or developing countries, it has been for the weakest among them much more than the straw that broke the camel's back. Quite gone are the days when optimists could calculate (as one official of the British Treasury did in 1974) that OPEC's enforced increase of the price of oil would have the beneficial (anti-inflationary) effect (because of higher consumer prices) of taking money out of circulation, and that the oil-producing governments would have no choice but to lend back their surplus and unrequited cash, so that oil-consuming governments would be able to reduce their demands on their domestic money markets, thus making capital investment easier. In reality, the trends have gone the other way. Inflation has become more rapid, especially in the weaker economies, partly because of the effect of the increased price of oil on consumer prices generally, partly because of the success with which people have persuaded their employers that they should be insulated from the OPEC prices and partly because the recycling of funds has been less effective and efficient than was originally supposed. At the same time governments, especially the weaker governments, have found it harder, not easier, to borrow money, and have found it possible to do so only by forcing up interest rates. The turbulence of the New York money market since the beginning of this year is one symptom of the malaise. Another is the absurd truth that the British government's annual borrowing (estimated at £12,000 million this year) is just about enough to pay the interest on its accumulated debt.

Especially on the eve of a recession, nobody can be sure where this will end. The conventional wisdom is to suppose that both the oil producers and consumers have a common interest in the avoidance of calamity, but that cannot be the case. Most OPEC members (but Iran and Nigeria are probably exceptions) could manage well enough if worldwide demand for oil were sharply to decline. Although the surpluses they have earned in the past years are still largely in the form of paper money, unrequited by the transfer of goods and services and continuously being eroded by the depreciation of currencies, they may well calculate, on paper correctly, that they can always make good past losses by charging even more for future production. No doubt the rash of \$2 a barrel

increases in the past few months derives in part from such calculations.

Consumer resistance so far has been fitful. Several oil companies, especially Japanese oil companies, are, however, for the time being at least, declining to buy Iranian crude oil at \$35 a barrel. Time will tell whether Gulf Oil and Charter Oil eventually agree to pay the \$38 a barrel that Qatar is asking for contracts running from the end of April. One hapless lot of consumers will however have no choice. Customers of the British National Oil Corporation were told last week that they would have to pay \$36.25 a barrel for oil from the North Sea with effect from 20 May. It is not so long since British Prime Ministers, flushed with the success of exploration in the North Sea, were making jokes about applications for OPEC membership. The formalities of membership seem not to matter where prices are concerned. The technical explanation of this curious behaviour, the effect of which will be to take even more money out of an economy now well into a recession, is that common international pricing has been written into the British government's relationship with the North Sea oil producers. British taxpayers will nevertheless with justice ask how, and in what form, they will eventually enjoy the supposed benefits of North Sea oil.

Ironically, the most recent wave of increases in the price of oil has come at a time when oil is, if anything, in good supply. Thanks to the urging of the International Energy Agency, stockpiles of petroleum in most industrialised countries are getting on for a quarter of annual consumption. Marginal oilfields outside the OPEC states are being worked harder, while there has been a cheerful pause in the previously steady decline of proved reserves in the United States. At the same time, the oil-consuming countries have made some progress — but less than it was reasonable to hope for — in limiting their consumption and import of petroleum. In the European Community, for example, oil consumption was no greater at the end of 1979 than in 1973, before the whole upheaval began. But it is too soon to think that the international market in oil has become a buyers' market.

More could however be done by the oil consumers to take advantage of the present contradiction between the plentiful supply of oil and the increasing trend of prices. For a time at least, the oil companies might be persuaded to abandon their traditional practice of buying the bulk of the oil they need (and sometimes more than they can sell) on long-term contracts from the oil producers. What the oil consumers need is a market in which some commercial balance would be struck between supply and demand. In the past few years, Rotterdam has won itself fame and fortune as the centre at which crude petroleum is traded in this way, and it is hard not to think that oil-consuming governments have an interest in strengthening this device for deciding a commercial price for oil. The oil producers at any time most in need of cash could be counted on to provide the market with something to sell. Such a way of fixing prices rationally would be made enormously more effective if industrialised countries such as the United States and Britain were to agree that some of the oil produced within their borders might also be disposed of in that way. Unfortunately, since 1973, the governments that might be able to help in such ways have moved in the opposite direction, putting self-sufficiency before self-interest.

No clever organisation of an international market in petroleum can provide more than a short-term solution to the problem of



increasing oil prices. A decision by, say, Saudi Arabia to reduce production by two million barrels a day could speedily put a crimp in the most efficient market. This is why the only long-term solution is that the oil-consuming countries should lessen their dependence on OPEC oil. So much has been clear since 1973, and is enshrined in the policies of most of the international bodies with interests in this field, the International Energy Agency and the European Community in particular. Alas, this is yet another field where declared policy and practice are far apart.

This is not to belittle what has been accomplished. In the past few years there has been a hopeful change in the pattern of energy consumption within the European Community, for example. Total energy consumption has grown less quickly than foreseen five years ago, not simply because economic activity has lagged behind the original estimates but because energy conservation has been a modest success (especially among industrial and commercial users). The supply of energy from sources other than oil has also, unfortunately, fallen behind the targets set in 1975. Both coal and nuclear energy production are unlikely, in the

coming decade, to do much to lessen dependence on OPEC oil (which will still account for nearly 50 per cent of European energy consumption in 1985). This, for Europeans, is too much for comfort. Last month, the European Commission did its best (without much success) to persuade the energy ministers of the Nine to look seriously at a series of proposals intended to make the European market in energy internally more efficient — common principles for pricing, taxation and the like.

In the United States, as always more fortunate, the benefits of President Carter's tardy and apparently reluctant freeing of the United States energy market have still to appear. As the letter from Dr Alvin Weinberg illustrates (page 382), it will be some time before even Americans acknowledge that the problem of energy prices is a serious problem. The stakes, at least for oil consumers, are high — nothing less than the perpetuation of a way of life they have grown fond of. The cost of winning the gamble may seem high — a reactor here, a strip-mine there. In reality it is not. For the only way of bringing down the price of oil is for the oil consumers to show that they could, if need be, do without it.

How not to foster new technology

The present British Government seems to be making a thorough muddle of its predecessor's plans for setting up a microcircuit industry in the United Kingdom. More than two years ago, when politicians all at once found out about micro-electronics, the then Labour Government launched a flurry of new (and expensive) programmes — schemes for helping industry at large to become familiar with the new technology, for spreading the word through the educational system, for compiling lists of consultants on information processing and the like. The most striking part of the hastily-assembled package was a decision to invest £50 million in a company called INMOS, newly formed by people with American experience, whose strategic role was to be the design, development and manufacture within the United Kingdom of an advanced range of microcircuit devices. The channel for the Government's investment was the National Enterprise Board, best known as the public shareholder in a variety of companies which successive governments have chosen to rescue from one kind of trouble or another with public funds. Sensibly enough, the NEB decided that INMOS could not have all of the promised £50 million in one cheque — half was paid over in 1977 on the understanding that the second half would be available when the design and development phase of the enterprise had been completed.

Since then, a great deal has changed. There is a new British Government, suspicious (to say the least) of the function of the NEB. The NEB itself has changed dramatically — in November 1979, the entire board chose to quit when the Department of Industry (to which the NEB is responsible) decided to remove the aero-engine manufacturer Rolls-Royce from its field of interest. And the new government, superintending a board whose mere existence is an embarrassment, is more desperately short of taxpayers' money than it had thought possible.

INMOS, it appears, has been an unlucky victim of these changed circumstances. Last summer, when the design of two microcircuit devices had been completed (physically in the United States) the company asked the NEB for the second tranche of funds. In October, soon before the board's decision to quit *en bloc*, the plan was approved, but a formal recommendation was not passed to the Department of Industry (which keeps the cheque-book). In December, therefore, the newly-constituted NEB reconsidered INMOS's proposals, approved them formally and recommended that the Secretary of State for Industry, Sir Keith Joseph, should write a cheque. Since then, nothing has happened.

To judge from Sir Keith Joseph's exhortations to people in California last week, neither he nor the government to which he belongs has cooled on microprocessors. By all accounts, he was urging American manufacturers to establish themselves in Britain, dangling before them the attractions of tax-breaks and

low wages (on paper at least). Nothing appears to have been said about INMOS and the second £25 million.

The truth is, of course, that the British Government is morally committed to provide the second half of the investment estimated as necessary to launch INMOS and its first few products. Nobody claims that the company has failed to meet the conditions laid down when the formation of the company was encouraged by the then government. So far, two sophisticated microcircuit components have been designed and tested — a 16 K static memory and a 64 dynamic memory. Each of them is likely to interest computer manufacturers. There may be doubts about the capacity of a new and relatively small company based in Britain to sell its wares effectively in the international markets to which it must be looking. Yet having helped INMOS so far, it would be folly now not to provide it with the funds it needs to set up its manufacturing plant. To fail to do so would be disreputable. To delay is to load its dice against the company.

None of this implies that Sir Keith Joseph is not right to be agonising (as is said to be his wont) about the good sense of the mechanisms he has inherited for using public funds to sponsor technological innovations. The National Enterprise Board itself is an awkward legacy of Sir (then Mr) Harold Wilson's promise in 1963 that, if elected, he would create a second industrial revolution with "white-hot technology". The surviving marks of that period are a few aluminium smelters scattered through Britain, a number of industrial companies too big for their own comfort and the NEB, the descendant of the old Industrial Reorganisation Corporation.

Throughout this period (as before it began), governments and British governments in particular have demonstrated that they are uncommonly bad at backing technological enterprises. The reasons are simple. Civil servants are not equipped to make judgements of what will work and also sell. They and their political masters are prone to attempt too much in their sponsorship of innovation — they also seek to prop up ailing companies or to provide jobs in depressed parts of the country for reasons which have nothing to do with innovation. It would be entirely proper if the Department of Industry were to seize this opportunity for a radical re-examination of how best to foster new industry. The most important ingredient of a new policy would be to foster circumstances in which people who know what's what find it profitable to back their hunches, but there is much to be said for the British Government deliberately replacing some of its in-house research and development by contracts let to private industry. The National Enterprise Board could be abolished without much harm — and that, of course, may happen anyway if the British Government insists that it should act as an agent for going back on a promise, however unwise that may have been. □

One way ahead for British biotechnology?

BRITISH biotechnology may yet survive. A plan by three renowned molecular biology laboratories for setting up in Britain a commercial biotechnology venture is being considered by several agencies of the British government.

The laboratories concerned are the MRC Laboratory of Molecular Biology at Cambridge, the Laboratories of the Imperial Cancer Research Fund in London and the Cold Spring Harbor Laboratory on Long Island in the United States. The three principals are Dr Sydney Brenner, Dr Walter Bodmer and Dr J. D. Watson, the respective directors of the three laboratories.

The essence of the plan is to establish a small organisation in Cambridge, initially for the preparation and supply on commercial terms of monoclonal antibodies, principally for use in biomedical investigations.

This would involve exploiting a technique originally developed by Dr César Milstein of the Laboratory of Molecular Biology, Cambridge, in which lymphocytes producing antibodies of a specific kind are fused with cells of cancerous lymphoma tissue.

After suitable selection procedures, the outcome can be a line of cells with the vigour of lymphoma cells which nevertheless produce the specific antibody prolifically.

This particular plan has emerged over the past year, and thus in its origins predates the Spinks Report on biotechnology, published at the beginning of April (*Nature*, 10 April and 24 January). That urged that the research councils and the public sources of venture capital in Britain, especially the National Research Development Corporation and the National Enterprise Board, should take steps to promote a British biotechnology industry. Both organizations have announced their interest in the field (*Nature*, 29 May).

The production of monoclonal antibodies has presumably been chosen as a first objective because it could be applied immediately, thus providing a new venture company with some bread and butter. The initial need of capital would be correspondingly modest.

The next steps to be taken in the setting up of a commercial venture are by no means obvious. The Medical Research Council, the obvious sponsor of such a venture, is precluded by its terms of reference from risking the loss of money. The NRDC is used to dealing with particular products and inventions, not with backing companies as such.

On the face of things, the National Enterprise Board is constitutionally better able to provide the backing a venture like this would need. One intriguing possibility is that the two private laboratories — Cold Spring Harbor and the Imperial Cancer

Research Fund — would be able to provide some of the capital if their boards of trustees agreed.

A spokesman for the National Enterprise Board declined this week to say whether his board was considering this particular proposal, but reaffirmed the organization's concern with biotechnology. It is, however, understood that

Research councils

Disquiet over NERC succession

THE appointment of Sir Hermann Bondi as full-time chairman of the UK Natural Environment Research Council (*Nature*, 15 May), although widely welcomed, is also the cause of consternation. At its meeting last week (28 May), the Council was sore that it had not been even formally consulted and concerned that the forced resignation of Mr R J H Beverton, secretary of the council for the past sixteen years, might be misinterpreted.

Members of the council agree with the chief objective of the change at the top — that NERC should be put on the same footing as the other research councils, with the responsibilities of chief executive and official accounting officer combined in the same person. Hitherto these functions have been the responsibility respectively of the Secretary and the Chairman, for the past three years Sir James Beament, head of the department of applied biology at the University of Cambridge.

What irks the council and dismays some of the directors of NERC research establishments is that when Sir Hermann's candidacy for the job became known to the Department of Education and Science early in April, both Beverton and Beament were presented with a *fait accompli* and, in effect, invited to go quietly. This, in the event, they both agreed to do.

The urgency of the decision stemmed from Sir Hermann Bondi's impending retirement as Chief Scientific Adviser to the Department of Energy with effect from

Beverton out . . .



some news of the NEB's interest in the field will be forthcoming within weeks rather than months.

Much may depend on whether biotechnology is able to avoid the Catch-22 problem which has beset micro-electronics (see page 346, this issue) — if it's not good, it's not worth backing; if it is, why doesn't the market step in?

1 October, required by Civil Service rules on age. The NERC Council was left with the impression that if the succession at NERC had not been settled quickly and to his satisfaction, Bondi would have taken his undoubted talents elsewhere.

The past few years have seen something of a change in the character of NERC's programme. Although NERC continues to spend its own funds on a programme of basic research not markedly different from in the past, funds awarded to NERC by government departments for contract research ('Rothschild money') have increasingly emphasized energy-related problems — radioactive waste disposal and physical oceanography related to the exploration of the North Sea, for example.

Although the division of the responsibil-



. . . Bondi in

ities of chief executive and accounting officer is agreed to be the long-term reason for change, the move of the NERC headquarters from London to Swindon has complicated the problems of management in the past two years. Sir James Beament, based at Cambridge, has been spending two days a week on NERC business but has been hard-pressed to divide this time between London and Swindon.

Even so, there are many who consider that some role could have been found in the new organisation for Mr Beverton, who has only two years to go to his normal retirement date.

The council was at pains to emphasise at

its meeting last week its appreciation of Beverton's service to NERC and its hope that the Civil Service would find a worthwhile job for him to do in the next few years. The council was especially at pains to emphasize that Beverton's sudden departure betokened no misconduct of NERC's affairs.

For the British research council as a whole, the incident has been a somewhat chilling reminder that their jealously guarded autonomy is, in the last resort, in the gift of the Department of Education and Science. In the week in which another government department (Energy) precipitated the resignation of the financial managing director of the British National Oil Corporation by the unwelcome appointment of a new chairman (without consultation with the board), that should not be a surprise.

Radiation

ICRP rules row

Washington

Fitting round pegs into square holes must seem child's play compared to the political difficulties of bringing US radiation exposure regulations in line with the current state of scientific knowledge.

In an unusual reversal of roles, officials of the US Nuclear Regulatory Commission have expressed reservations about new occupational exposure guidelines being prepared by the Environmental Protection Agency, claiming that they would result in an unnecessary relaxation of certain existing restrictions.

The dispute centres on recommenda-

tions for revising occupational exposure to radiation proposed three years ago by the International Commission on Radiological Protection. These have been accepted as the basis for regulation by the Commission of the European Economic Community, but remain the centre of fierce controversy in the United States.

The method for calculating maximum exposure levels proposed by the ICRP in its report known as ICRP 26 is widely accepted as a major advance and as reflecting the best 'state of the art'. For example, it allows for joint consideration of the effects of internal and external doses of radiation, previously considered separately.

Furthermore it shifts the basis for calculating maximum exposure levels from consideration of 'critical organ' doses — using the maximum acceptable exposure to organs most susceptible to a particular radionuclide — to a method which calculates a general level of risk by integrating the weighted risks posed to various parts of the body.

The advantage of this approach is that it includes the risks to organs other than those considered the most critical. The difficulty, however, comes from the need to adjust the specific figures placed on exposure limits.

Controversy has in particular focused on the ICRP's suggestion that the maximum integrated risk should be equivalent to that represented by the existing maximum whole body exposure of 5 rems a year.

The EPA, which is responsible for setting exposure guidelines to be followed by other agencies, has yet to issue formal proposals on revised exposure levels. But it has in-

formally sounded out the agencies on the use of the ICRP aggregated-risk methodology, based on a maximum organ dose of 30 rems a year.

Even this reduced exposure guide, however, has not been acceptable to some NRC officials. While supporting the ICRP methodology in principle, they argue that the result of meeting the overall risk requirement would be to permit an increase in permitted air concentrations for many radionuclides, in some cases by an order of magnitude.

The NRC officials, who say their arguments have been accepted as an interim position by the NRC commissioners, agree that such increased limits would not necessarily be harmful. But they argue that they would inevitably reduce the protection afforded to workers at licensed power plants and uranium mines — and that the nuclear industry apparently has little difficulty in meeting current standards.

EPA officials agree that adoption of these proposals would permit increased exposure to some radionuclides (as well as reducing exposure to others). But they insist that assessments should be based primarily on consideration of the overall risk, rather than merely the risks to separate organs.

"If someone gets cancer, it does not really make much difference to them which part of the body they get it in. We are trying to limit the amount of harm to people. That is not the same as limiting the dose in an abstract sense", Dr David Rosenbaum, director of EPA's Office of Radiation Programmes, said last week.

NRC officials have proposed a hybrid scheme under which exposure limits for individual radionuclides would be calculated both by the ICRP methodology and by the 'critical organ' technique using the same methodology but old dose limits, accepting whichever is the lower. But EPA is unenthusiastic about this approach.

The situation is complicated by each agency's desire to respond to outside arguments. The EPA, for example already faces challenges by nuclear companies on its proposal that public exposure outside a nuclear facility should not exceed 25 millirem.

At the same time various public interest groups are using the uncertainties in the scientific evidence to petition the NRC to reduce the present 5 rem occupational exposure limit by an order of magnitude. Several trade unions are also planning to press the EPA not to introduce the ICRP 26 scheme without major modifications.

Given all this activity, publication of the proposed guidelines is now unlikely before the autumn, with a period for public comment to be followed by a series of public hearings next year. These promise to be lively; particularly if the Administration changes to one more concerned to minimise regulatory restraints on the growth of nuclear power. **David Dickson**

Soviet heavy neutrinos

BARELY a month after a 'Science Day' speech in which Anatolii Aleksandrov, President of the Soviet Academy of Sciences, suggested that the Soviet Union should make itself as independent as possible of western scientific results, a team of physicists led by Academician Valentin Lyubimov, has repeated the claim of Dr F. W. Reines to have established that neutrinos have mass. This has now been announced by the Russian news agency Tass. Reines, from the University of California at Irvine, described to the Spring Meeting of the American Physical Society last month the latest in a series of experiments at the Savannah River reactor in which the relative importance of the charged and neutral currents in the interaction of reactor neutrinos with deuterons was measured. The neutrino mass deduced is the equivalent to a few electron-volts.

The Russian work now referred to is based on a different method. The team concerned, from the Moscow Institute of Theoretical and Experimental

Physics, is said to have analysed the spectrum of electrons in tritium decay, deducing the mass of the neutrino from the shape of the spectrum. The discovery was announced in a report delivered to the Presidium of the Soviet Academy of Sciences.

Commenting on it, Academician Yakov Zel'dovich observed that the result could produce major changes in current cosmological concepts and possibly raise once again the question of the existence of a cosmological constant, first mooted by Einstein in 1917.

Reporting the discovery, the Tass agency said that the existence of 'heavy' neutrinos solves a number of existing paradoxes, including the question of the missing mass of galaxies and the measured deficiency of solar neutrinos from the sun. Tass claimed that it also appeared to confirm the model of solar neutrino flux proposed by Academician Bruno Pontecorvo involving the inter-conversion of different neutrino types.

Vera Rich

Research application

Hungary expects

Budapest, May

The Hungarian Government is hoping to reshape the balance between academic and applied research without reducing the total number of research jobs. This was the burden of a statement by Mr Jozsef Marjai, one of the deputy chairmen of the Hungarian Council of Ministers, at the Investment Goods Spring Fair held here from 23 to 28 May.

Mr Marjai promised that there will be no reduction of the number of research jobs, and said that there may be even more jobs available for scientists and technologists. "But we cannot promise that everyone will stay in the same job."

Mr Marjai's remarks, however, echo a major dilemma in Hungarian science planning. At present, science in Hungary has a top-heavy structure with, it is generally agreed, too many research institutes for a country of 10 million people to support. Last month, Janos Szentagothai, president of the Hungarian Academy of Sciences, told the General Assembly of the Academy that time and energy could no longer be wasted on 'insignificant and middle-level' basic research. Plans are now being formulated for the network of research institutes to be modified to serve 'social and economic needs'.

There is little doubt that many young scientists are reluctant to leave the academic seclusion of their research institutes. Even the show-piece Babolna collective farm has found it difficult to recruit a qualified agricultural economist. Young biology graduates joining the Plant Protection Service speak of their fieldwork as if it were a kind of purgatory. Hungary needs scientists to work in agriculture and industry, and while the planners so far have been unwilling to deny promising students a few years post-graduate research, the view is growing that the country simply cannot afford to give them a niche in some institute for life.

The problem can, to a certain extent, be

ameliorated by directing research talents not to the needs of the Hungarian economy but to export. More than half of the Hungarian GNP comes from exports, and one pavilion at the Budapest Fair was used by the Academy of Sciences, Ministry of Education and National Committee for Technological Development to show that academic research can sometimes yield practical benefits. The goods on display ranged from a laser theodolite to a fixed-bed denitrification column and from a computer-aided electromyograph to an electronic Braille writer. "We don't want over-specialization", explained Gyoergy Paris, science organiser of the Ministry of Education. "We make what foreigners want to buy."

Nevertheless, like most Hungarian scientists, he was not over-enthusiastic about the plans to direct more scientists to industry. "It is a pragmatic solution", he said, "and not good for research. Some people simply cannot do practical work. But we are a small country — and what else can we do?"

Hungarian planners, of course, are not unique among their Comecon partners in trying to integrate science into production. The Hungarians, however, place less emphasis on the socialist virtue of the process. Nevertheless, the Hungarian problem is exacerbated by the lack of natural resources and by a long tradition of academic elitism.

As in most Comecon countries, Hungary has developed a research structure based on Academy institutes which are independent of the universities, and, perhaps, too large for the country to support. Eight years ago, Bulgaria solved this problem by integrating the staff of the institutes of the Academy of Sciences back into the teaching structure of the universities. In the next few years, it seems likely that Hungary may be forced to adopt a similar solution.

During the past year there has been a major press debate, launched in May 1979 by an article by Professor Gyoergy Adam, former Rector of Budapest University, in *Magyar Tudomány* ('Hungarian Science'). The whole role of the university and its is already under discussion.

Vera Rich

High-energy physics

More of less money

Washington

American high-energy physicists are in a jittery mood. They are now faced with the prospect that the three main national particle accelerators may have to close down during the summer months as a result of Congressional budget cuts — and that there may be worse in store next year.

The immediate difficulty stems from a recommendation by the Appropriations Committee of the House of Representatives that \$8 million would be rescinded from the \$325 million already approved — and largely unspent — for the current financial year.

Half of this saving would come from delaying construction costs of the 400 GeV Isabelle Project at the Brookhaven National Laboratory, with relatively little impact expected over the full seven-year period of the project. But the remainder would include \$1 million taken from physics research, \$2 million from facility operations, and \$1 million from high energy physics technology.

The only way such cuts could be absorbed would be to stop all experimental work at the three national accelerator laboratories until the new financial year begins in October. Dr Leon Ledermann, director of Fermilab, said last week that the decision would be disastrous.

Construction of Fermilab's new energy-saver, designed to double the accelerator's energy to 1000 GeV by the use of superconducting magnets, is already biting deeply into the laboratory's operating funds. Further reductions could mean that the operation of the accelerator was halted entirely next year, so that the construction of the Tevatron could be made the laboratory's top priority, according to Dr Ledermann.

At Brookhaven, the proposed reduction in operating funds would mean that between eight and ten weeks of experiments planned for the summer would have to be put off. And the same would be true at the Stanford Linear Accelerator (SLAC) in California, where the new PEP collider has only just come into operation, but on which experiments could not be carried out until the autumn.

In an already tight financial year, the budget axe seems to be falling particularly heavily on energy research, partly because the energy budget is considered jointly by Congress with the budget for dams and other 'pork barrel' construction projects which carry considerable appeal to legislators in an election year.

The situation for next year remains cloudy, largely because the debate over how to balance defence and social expenditures within a balanced budget means that Congress has yet to agree on its own financial guidelines. However there are



Hungarian Academy — changing with the times?

several pointers around, and none of them is promising.

Thus although the Carter Administration recommended significant increases for high energy physics both in its original budget request in January and in the revised request in April, the Senate Budget Committee has proposed reductions in the whole of the science budget to make room for extra defence spending.

The equivalent committee in the House of Representatives has not been so harsh. But, like that in the Senate, it is suggesting that first priority within the science budget be given to the space shuttle; and has pinpointed high-energy physics as one area that might accommodate a 'pause in funding'.

More specific proposals have been made by the House Science and Technology Committee, which has recommended a high-energy physics budget for next year of \$6 million less than the President's revised request of \$354 million.

This cut could be absorbed relatively easily. Proposals from the House Appropriations Committee are expected to be more damaging. No decision has yet been made but it is widely reported that the whole of the energy research budget — including high-energy physics — should be kept at its current level for next year, with no increase to allow for inflation.

The implication of such a proposal, if accepted by Congress, would be to wipe out the 10 per cent increase which had been expected by the high-energy physics community.

The prospect of no growth for the energy research budget has already prompted a letter from Dr Frank Press, Director of the

Office of Science and Technology Policy, to the chairman of the Appropriations Committee emphasising the importance that the Administration attaches to the support of basic science.

But in a generally confused budget year, it remains unclear which direction Congress is likely to take. All that is certain is that the bleak outlook will be hanging heavily over physicists meeting this week in Woods Hole, Massachusetts, to chart a strategy for US high-energy physics over the next decade.

David Dickson

Astronomy

Britain bids for big time

BRITISH optical and radio astronomers have decided to bid for £15.5 million-worth of new telescopes — a 4.2 m optical collector and a 15 m millimetre-wave radio dish — to be constructed at the Roque de los Muchachos Observatory site on La Palma, in the Canary Islands. The request effectively mortgages all major capital spending by the Science Research Council's Astronomy, Space and Radio Board until the middle of the decade.

The proposal is now before the Advisory Board for the Research Councils as part of the SRC's 'forward look', and is within previously agreed guidelines for SRC expenditure. However, because the cost is more than £200,000, the proposal also has to be approved separately by the Secretary of State for Education and Science. A decision is expected within a month. The

Netherlands is also involved; the National Research Council (ZWO) is considering a 20 per cent participation in the new instruments.

The 4.2 m light collector is designed to provide high resolution imaging of distant faint sources, comparable with that of the Hale telescope at Mt Palomar, but with considerably better seeing — the best, in fact, in the northern hemisphere. Combined with the UK Infrared Telescope (UKIRT) on Mauna Kea, Hawaii, and the complementary 1 m and Isaac Newton 2.5 m telescopes already destined for La Palma, the 4.2 m would double the amount of guaranteed telescope time available throughout the world to UK optical astronomers.

This estimate takes account of the Roque de los Muchachos agreement, which allocates 20 per cent of the observing time on the telescopes to Spain and 5 per cent to collaborations among other users of the observatory. These are at present Denmark and Sweden; but a site has already been reserved for a French 90 cm solar telescope, and there is a possibility that Italy will join with its own 3.5 m telescope.

The Italian move stems from its recent proposal to join the European Southern Observatory in January 1981, alongside Germany, the Netherlands and Sweden, at ESO's La Silla site in Chile. Part of the price of admission — which is still under negotiation — would be to provide a 3.5 m blank to provide a second major ESO light gatherer (a 3.6 m telescope is already in place), and Italian astronomers are considering splitting a large blank into two halves, one for La Silla and one for La Palma. However, their proposal depends on gaining Italian parliamentary support, which is often slow in coming.

The millimetre-wave radio telescope faces greater competition. The United States has had an 11 m instrument reaching down to 2 mm wavelength in place at Kitt Peak for a decade; and France and Germany are constructing a similar dish, and a millimetric interferometer, within the Institut pour Radioastronomie Millimétrique (IRAM) whose headquarters are in Grenoble.

The United Kingdom rejected participation in IRAM because it involved setting up a new and expensive administration, because European salaries would have to be paid, and because it was against the SRC's policy of disengagement from large autonomous research centres.

The proposed millimetre radiotelescope would be complementary to the infrared telescope at Mauna Kea, where the seeing is as good as at La Palma. The infrared telescope, which can operate at most wavelengths between the near-visible and 1.5 μm (with the help of helium-cooled bolometers) cannot provide detailed spectral information. The proposed radiotelescope, using tuned-crystal detectors, will on the other hand be able to provide the profiles of molecular emission

Existing major international optical telescopes

Diameter (m)	nationality of operator	location
6.00	USSR	Mt Semirodriki
5.08	USA	Mt Palomar (Hale)
5.00	USA	Las Campanas, Chile
4.50 (6 × 1.8)	US (multimirror)	Mt Hopkins, Arizona
4.22	UK (infrared)	Hawaii
4.01	USA	Kitt Peak
4.01	USA	Cerro Tololo, Chile
3.94	Anglo-Australian	Siding Spring
3.66	ESO	La Silla, Chile
3.66	Franco-Canadian	Hawaii
3.05	USA	Mt Hamilton (Lick)
2.72	USA	Ford Davis, Texas (McDonald)
2.64	USSR	Crimea
2.60	USSR	Armenia
2.59	UK (Isaac Newton)	La Palma, Canaries
2.57	USA	Mt Wilson
2.54	USA	Las Campanas, Chile
2.29	USA	Kitt Peak
2.24	USA	Hawaii
2.20	W. Germany	Calar Alto, Spain
2.15	USA	Kitt Peak
2.15	Argentina	San Juan, Argentina
2.08	USA	Fort Davis, Texas (McDonald)
2.00	USSR	Chemakha

lines at wavelengths greater than 0.8 mm. It will also provide Britain's radioastronomers — whose long lead in radiointerferometry has been broken by the construction of the US Very Large Array — with access to at least one world class instrument.

Robert Walgate

Ariane

Half way to the Devil

EUROPEAN hopes of commercial competition with the delayed American space shuttle have been called into question by the failure on 23 May of the second launch test of Ariane, the European three-stage 1,750 kg payload launcher. The European Space Agency, responsible for Ariane, has always held that two successful flights in the four-flight test series (the next tests are due in November this year and February 1981) would be counted a success; but what matters is whether potential clients will take the same view. They will no doubt now be on their guard.

The rocket fell into the sea less than 10 km from its launch site in Kourou, French Guyana. It now lies in muddy waters some 7 to 10 metres deep between Kourou and the former French penal colony, Devil's Island. Two ships are searching for it, one with sonar and the other with drag-nets. If the rocket is found, it should be recoverable.

The fuel tanks were destroyed by an automatic device, but the propulsion bay — the collection of four combustion chambers where the critical fault occurred — is expected to be intact. It may prove crucial in determining the cause of the accident.

Preliminary analysis of telemetric data has revealed that one of the four first stage engines began to 'flicker' 4.4 sec after ignition, 1.1 sec after lift-off. The pressure in its combustion chamber oscillated at 1,000 Hz with an amplitude of 4 bars about its design pressure of 54 bars, and the exhaust gases turned yellow, indicating inefficient oxidation of the fuel. By 6 seconds the fault appeared to have corrected itself.

At 28 seconds the fault recurred for an instant, and then the temperature began to rise steadily in the propulsion bay from 24°C to 56°C. At 64 seconds the temperature jumped to 100°C and the combustion chamber pressure dropped to 10 bar. Ariane started to roll. By 104 sec the roll had reached 10 rpm, and two other engines lost pressure; the third followed at 108 sec. The self-destruct systems then decided that Ariane had had enough, and blew out the fuel tanks.

A similar loss of pressure was detected just after ignition of the Ariane engines on the first launch test in November 1979; but

this loss was detected soon enough to abort the launch. At a subsequent attempt, lift-off and launch were successful, and the earlier fault was put down to instrument error. There may now be doubts about whether this was the case.

The Ariane first stage engines are derived from those of an earlier French rocket, 'Diamant' — and this rocket also experienced catastrophic losses of power during its development. However, 44 ground tests of the Ariane four-engine assembly showed power loss only once.

The fault probably traces back to a fluid or chemical instability in the injector, the 'carburettor' that injects fuel and oxidant into the combustion chamber; and the question arises whether the instability is triggered by a mechanical fault in the injector, or is simply an inherent instability due to a design error. If the latter, a further series of ground tests will be necessary before Ariane's third test flight, and the money available for those tests is limited.

Another possibility being considered by Ariane engineers is that the geometry of the exhaust deflectors at the launch pad led to

an acoustic effect which in turn induced the instability. The deflector geometry is completely different at Kourou from the geometry adopted in the ground-based tests.

Robert Walgate

Pesticides

EEC directive disputed

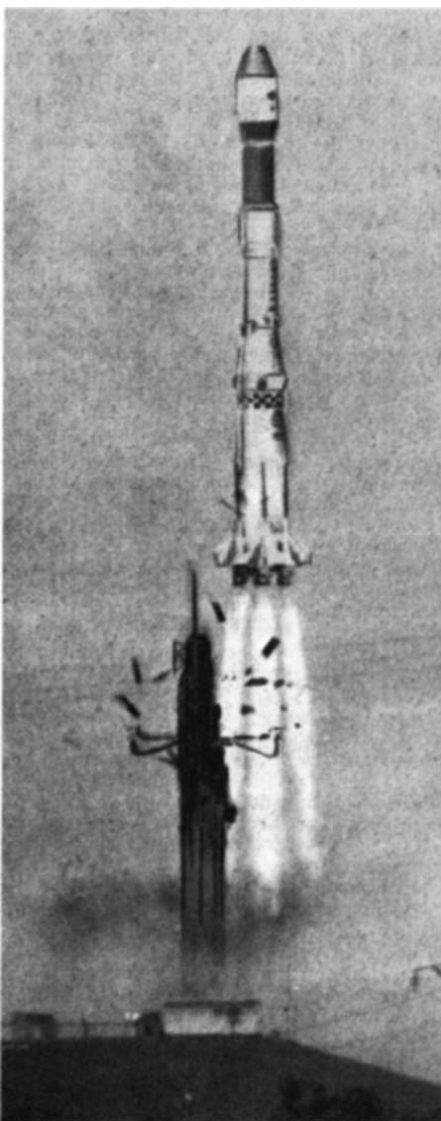
THE long-standing argument over the basis for environmental standards between some European governments and the European Commission now appears to have spread to the consumers' associations. Thus last week (27 May) the Consumers' Association, the London-based consumer group, published a criticism of the draft directive on the regulation of pesticides in foodstuffs originally promulgated in 1976.

The CA's argument is based on a comparative study carried out in the past three years in West Germany and the UK. The nub of the issue, according to the report 'Pesticide Residues and Food' (Consumers' Association, 14 Buckingham Street, London WC2) is whether the regulation of pesticides in the human diet is best accomplished by fixing maximum concentrations of permissible pesticide residues in foodstuffs appearing on the domestic market.

The EEC directive proposes that there should be maximum permitted levels of certain pesticides (particularly the organochlorines) in foodstuffs, and that it should be the responsibility of national governments to sample foodstuffs regularly, analysing them for their pesticide content. This is for practical purposes the system now in operation in West Germany.

By contrast, the British system is based on a voluntary scheme for the approval (by the Ministry of Agriculture, Fisheries and Food on the recommendation of an expert Advisory Committee) of pesticides offered for sale in the United Kingdom and on estimates of what foods people eat. The CA joins with other critics of the system in urging that the approval scheme should be made statutory, but otherwise comes to the conclusion that this scheme has adequately served its purpose of keeping pesticide concentrations in human blood and tissues within acceptable limits.

One of the CA's chief arguments in favour of the British system of control is that the German system is much more expensive. But the report, while recognising that harmonisation of environmental standards is necessary in the interests of European free trade, argues that it would be inequitable that all members of the EEC should have to adopt the most stringent (and cumbersome) of the control procedures now in force.



The Ariane launch, just as the first of the four engines began to flicker.

CORRESPONDENCE

Reactors away

SIR,—We nuclear technologists have plenty of reason to consider ourselves unloved and misunderstood. On 20 March in Middletown, Pennsylvania, not far from Three Mile Island, some 400 angry local residents bitterly attacked officials of the Nuclear Regulatory Commission over the proposal that the 60,000 curies of ^{85}Kr still trapped in the containment vessel should be released. This is a necessary first step in decontamination of Three Mile Island. The estimated maximum dose to any resident of Middletown caused by this release would have been 11 mr beta skin dose, 0.2 mr whole body gamma dose — the latter being of the same order as the dose received in a transatlantic flight.

The local people at the meeting were filled with bitter resentment and mistrust of NRC, as well as a deep, almost hysterical fear of low levels of radiation. I myself do not see how nuclear energy can survive in the long run — or possibly in the short run — if the public cannot distinguish between say, a millirem and a million rem.

It is for such reasons that I have urged that reactors be confined, permanently, to a relatively few and rather remote sites. C. Burwell, J. Ohanian, and J. Lane of the Institute for Energy Analysis have shown that 600 large reactors — possibly the number that will be built in the United States by the middle of the next century — could fit on about 100 large sites. Most of these could be expansions of the existing nuclear sites which, surprisingly, are already rather remote.

In heavily populated Europe, there are few remote sites — although even in Denmark such sites can be found on Zealand. Nevertheless, even if the sites are not remote, there are many other advantages of clustered siting — better organization on site, security and internal lines of transport for example. It is significant that almost one-half of all the nuclear power to be generated outside the US by 1985 will move from sites containing four or more reactors; three sites have eight or more reactors on them.

The environmental hysteria displayed at Middletown is not confined to low levels of radiation. The litany of incidents involving toxic effluents grows longer each day — Seveso, Mississauga, Love Canal. Hardly a nightly TV news broadcast in the United States goes by without another account of people suffering maladies attributed to low levels of industrial toxicants.

I am therefore much taken by Dr Pietro Cappuro's proposal in *Clinical Toxicology* 13, 325 (1979) "that industrial plants be concentrated in groups, at locations where natural factors such as wind, land contour, and water flow provide rapid dilution of toxic effluents". The notion of rather remote, permanent industrial enclaves is not new. It is striking that many of the same arguments that support remote, confined, and permanent siting for nuclear energy are being adduced to support a siting policy for polluting industry in general.

An important element in the proposal for nuclear siting is that each site should be committed, if not in perpetuity, then for a very long time. Low-level radioactive wastes could thereby be handled on site, as is now being done at the large Bruce site in Canada and at the seven TVA nuclear sites. This same imputation of permanence could be exploited for non-nuclear wastes as well. If the sites at which industrial activity continues are also used for disposal of low-level chemical wastes, fiascos such as that of Love Canal, where toxic wastes were nobody's responsibility,

would be dealt with rather automatically.

Like nuclear energy, the potentially dangerous chemical technologies demand long-term attention to detail and organizational integrity in return for the benefits they confer. It is striking that a recipe for living with the nuclear Faustian Bargain — to confine the enterprise to relatively few, permanent sites — is now recognized as applying to other technologies as well. Perhaps the nuclear enterprise can find some consolation in the growing realization that it is not unique in what it asks of the society in return for the benefits it confers.

Yours faithfully,

ALVIN M. WEINBERG

Institute for Energy Analysis,
Oak Ridge, Tenn., U.S.A.

Parkinson's trees

SIR,—R. Moss (*Nature*, 285: 9) has provided a useful mathematical model to help explain the now well-established (if bureaucratically unpopular) scale effect that results in large organizations being less productive than small ones. Non-mathematical readers may find a simple ecological comparison easier to follow.

A tree may be appropriate model. The leaves represent the primary producers or scientists, the branches and trunk the support staff. The ratio of leaf to branch weight can be of the required order, averaging 12.6:10.2 in *Picea abies* (J. D. Ovington, *Advances in Ecological Research*, 1, 103 (1962)). Trunk weight is about ten times the leaf weight but is very variable, depending perhaps on how high the tree canopy has to be elevated to reach the light.

Just what size a research group should be for maximum efficiency has been the subject of surprisingly little research, and that mainly by non-scientists. The tree analogy suggests we should consider separately the best size for a research unit (branch size), department (tree size) and national research body (forest size). The last will be directly related to the size, resources or needs of the country, but there is no reason why tree size would be related to forest size. A research department will have an optimum size and structure depending on its function, just as different species of tree will vary in size and leaf form; and a large country should establish many trees of this size, so that each can adapt to its local environment, rather than try to grow an extra large one.

If the pursuit of efficiency is desirable, useful comparisons of productivity could be made between branches of similar size on the same tree. Caution is needed to check that the branches have similar function and aspect (access to resources), while comparisons between branches on different species of tree (department or discipline) will be meaningless until some way is found of balancing their diverse aims or products. The most profitable comparisons are likely to be between branches on similar trees; for example, gamebird research units of state wildlife departments in America.

Administrators may argue that they are not dead wood but a structural device like a tree trunk to increase size, allowing successful competition with other ever-expanding trees, and enabling their research units to find a place in the sun. This, however, might be better achieved by cutting out the big trees and replacing them with smaller, more productive young ones, as any forester knows.

Before the axe falls there are the usual ecological provisos: agreement on the kinds of trees to fell and which to plant, retention of unusual trees whose genotype might be useful

in future, maintenance of variety and mixed age distributions for stability, and a reserve for such giant redwoods as the National Health Service.

Yours faithfully,

JOHN E. C. FLUX

230 Hill Road,
Belmont, Lower Hutt,
New Zealand.

Merrison's malady

SIR — That the Merrison Committee cannot resolve the malaise afflicting university research is clear enough (leading article, 22 May).

It is the readiness of our administrators to act as Healey-Joseph monetarist surgeons that has brought the malaise to this critical stage. The universities, in shedding technical and research posts, or equipment renewal and repair costs, have expected the research grant bodies to pick them up. The research councils, faced with impossible demands on static and contracting budgets, have resorted to financial limits apparently "calculated to ensure that even the best projects will not quite succeed" and to arbitrary time limits (such as the SRC's new ban on individuals holding an RA post for more than three or, exceptionally, six years).

Partly because we lack political muscle and partly because research jobs are regarded as transitory, contract researchers are being treated as optional extras — hard luck on personnel employed for years on soft money or unable to find a British university home to develop possibly outstanding projects. There are, however, some 10,000 contract workers in universities (Association of University Teachers figures) compared with 30,000 teaching staff. A proportion of university research of the order of 40 per cent might fairly be attributed to us.

Quality is relevant too. Experienced researchers are invaluable and irreplaceable for certain projects. Six or ten-year space research projects cannot be run by a succession of three-year contract researchers. To get round the SRC time limit, special dispensations for 'project managers' are currently being sought; but such subterfuge cannot meet the need for experienced instrument designers and data analysts. Already some space projects are threatened with run-down. Expensive equipment will be unused and expensively accumulated data will remain in store.

As you say, the Merrison joint committee between research councils and the UGC cannot be expected to discover a solution for the distribution of severely reduced funds. With only top academics and administrators, and no representatives of researchers or their trade unions, how could the seven-man (all male) working party invent an acceptable bargaining structure?

Surely the aim should instead be to analyse the malaise and conceive a holding operation; to persuade the UGC that it should not and cannot meet Government targets by cutting equipment grants, but must restore them with proper compensation for inflation, and to persuade the research councils to keep alive university laboratories and research teams.

Research itself will only suffer more from further surgery and hacking off weaker and less glamorous sectors. The holding operation should aim to maintain the successful integration of research in British universities until the government which conceives targets in monetary terms alone can be changed.

Yours faithfully,

M.K. WALLIS

University College, Cardiff, UK.

NEWS AND VIEWS

Phencyclidine

from Solomon H. Snyder

NEXT to marijuana phencyclidine, commonly known as PCP, is now the most widely abused street drug in the United States. In recent years PCP has received widespread attention because of the violent, homicidal and suicidal behavior of users. From the point of view of psychiatrists PCP is one of the most fascinating psychotropic drugs as the psychosis it elicits may provide the best available drug model of schizophrenia. For the pharmacologist PCP is a very unique psychoactive substance whose mechanism of action poses novel challenges.

Since PCP is often sold as tetrahydrocannabinol (THC), the principal psychoactive ingredient in marijuana, mescaline or LSD, it presumably mimics to a certain extent these drugs. PCP is widely sold in combination with other drugs such as barbiturates, heroin, cocaine, amphetamine, methaqualone (a barbiturate-like hypnotic), LSD and mescaline. Some of the street names for PCP include angel dust, crystal, elephant or horse tranquilizer, killer weed, super weed, monkey dust, Peace pill, goon, surfer and scuffle.

Phencyclidine is a cyclohexylamine (see figure). Large numbers of PCP analogues have been synthesized. Interestingly, at least six of these analogues are sold illicitly on the streets of American cities, a situation which is unprecedented among drugs of abuse. Besides PCP, whose chemical structure is 1-(phenylcyclohexyl) piperidine, five other PCP analogues have been widely distributed, namely PCE (N-ethyl-1-phenylcyclohexylamine), TCP (1-(1-2-thienylcyclohexyl) piperidine), PHP (1-(1-phenylcyclohexyl) pyrrolidine), PCC (1-piperidinocyclohexanecarbonitrile), and ketamine (2-o-chlorophenyl)-2-methylamine cyclohexanone).

What are the psychic effects of PCP? Its behavioural action is best understood by reviewing its history as a clinical anaesthetic and then as a drug of widespread abuse. PCP was synthesized by chemists at

the Parke Davis Drug Company at the end of the 1950s¹. In animals it displays some stimulant effects but also affects motor coordination producing a 'drunken' state. When administered to monkeys a state of apparent tranquility appears, which in larger doses develops into general anaesthesia. Because of what seemed to be serenity in the monkeys, the brand name of 'Sernyl' was introduced. Small doses of PCP in humans produce a drunken state with numbness of the extremities reflecting analgesia and even anaesthesia. In sub-anaesthetic doses human subjects seem to be in a state of sensory isolation with their eyes wide open, but unresponsive to the environment.

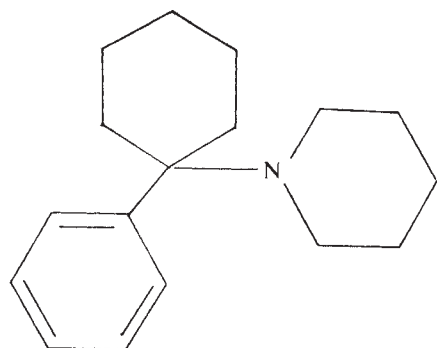
The drug appeared to be a relatively safe surgical anaesthetic, because it could produce anaesthesia with profound analgesia in the presence of normal reflexes of the pharynx and larynx. Moreover, in contrast to many anaesthetics whose toxicity is associated with respiratory depression, PCP stimulated both the respiratory and cardiovascular systems. Its only drawback was that when emerging from anaesthesia patients described confusional states, vivid dreaming and hallucinations. Because of these side-effects, PCP was withdrawn from use in humans, though it is still employed commercially as an animal anaesthetic. Ketamine, a PCP derivative which is shorter acting and has somewhat fewer side-effects, has replaced PCP for human use in surgical anaesthesia, though ketamine is also widely abused.

It is unclear just what aspect of the PCP experience attracts drug abusers. Interviews with large numbers of drug users suggest that the sedation, sensory isolation, seeming "drunkenness" and hal-

lucinations are sought after. However, together with the apparent sensory isolation comes a feeling of depersonalization which can be terrifying to patients. Indeed it is these subjective changes which make PCP an impressive drug model of schizophrenia. Patients describe a loss of the ability to distinguish self from nonself, a dissolution of ego boundaries and a sense of unreality². The disturbance in thinking has been described as "loosening of associations", "overinclusive thoughts", "concreteness", "delusional thinking", and "general disorganization", all strikingly reminiscent of the thought disorder of schizophrenics.

The most impressive evidence that PCP psychosis resembles schizophrenia is the fact that drug users have been mistaken by experienced psychiatrists for schizophrenics before obtaining the history of drug use. A particularly dramatic instance occurred in Washington, D.C. during the fall of 1973 when the admission rate for what appeared to be unusually long, severe and treatment resistant initial schizophrenic psychoses suddenly tripled in a community mental health center³. It was some time before the physicians recognized that these patients were suffering from PCP psychosis. Unlike the psychoses from psychedelic drugs such as LSD, which generally last only about 8-16 hours, PCP psychosis lasted for periods up to two weeks. LSD psychosis can be readily distinguished from schizophrenia. Schizophrenic hallucinations are auditory, while individuals receiving LSD generally experience only distortions in visual perception. With PCP, subjects may hear voices and display autistic and delusional thinking resembling that of schizophrenics. They may display the formal thought disorder of schizophrenics with a loosening of mental associations and a "blocking" of mental processes³. Another link of PCP to schizophrenia comes from studies in which PCP was given to hospit-

Solomon H. Snyder is Distinguished Service Professor of Pharmacology and Psychiatry, Departments of Pharmacology and Experimental Therapeutics and Psychiatry and Behavioural Sciences, John Hopkins University School of Medicine, Baltimore.



The chemical structure of phencyclidine.

alised schizophrenics². LSD produces no more severe effects in schizophrenics than in normal subjects. By contrast, schizophrenic symptoms are greatly exacerbated for up to several weeks following PCP administration.

One apparently unique aspect of PCP psychosis is its frequent association with aggressive behavior. Users feel that they have superhuman strength and invulnerability. More cases of homicide and suicide are associated with PCP use than with any other drug of abuse.

How might PCP exert its remarkable psychic effects? Though PCP has anticholinergic atropine-like effects⁴, these are much too weak to account for its behavioural actions. Actions at selective sites in the brain is apparent from regional metabolism studies with 2-deoxyglucose. Behaviourally active doses of PCP selectively increase 2-deoxyglucose uptake in the hippocampus, a portion of the limbic system which regulates emotional behavior⁵. A molecular locus for such an action is suggested by findings that PCP potentially affects ion channels at nicotinic cholinergic receptors^{6,7} which are abundant in the hippocampus. It is even possible that the seemingly great muscular strength and aggressivity of PCP users involves such effects at cholinergic receptors in the neuromuscular junction⁸.

A potentially powerful new approach to characterizing PCP actions stems from two independent reports identifying PCP

binding sites in the brain which may be unique PCP 'receptors'^{9,10}. PCP analogues whose affinities for the binding sites varied over a 20-100 fold range showed parallel variations in their disruption of the ability of rodents to balance themselves. Clearly the identification of specific PCP receptors would greatly assist in the understanding of how this drug and related agents act. It is possible that there are endogenous PCP-like substances analogous to the enkephalins, the normal neurotransmitters associated with opiate receptors^{9,10}.

Drug receptor binding studies can be deceptive because of artefactual binding sites masquerading as true 'receptors'. An artefactual binding effect was suggested by studies showing that the filters used in binding studies themselves bind radiolabeled PCP¹¹. Moreover, if the brain tissue is boiled, its PCP binding properties remain essentially unaltered. Relative potencies of several PCP derivatives are the same at binding sites on intact brain membranes, boiled brain membranes or filters with no tissue. This suggests that PCP binding sites are not in

fact true receptors but merely reflect saturable binding to nonspecific sites on filters or tissue. Such saturable but artefactual binding is well known. Thus, certain filters and lipids can bind opiates in a stereospecific fashion, with optical isomers displaying the same relative potency at the opiate receptor¹².

To clarify these questions, both the French¹⁰ and American⁹ groups have performed a number of control studies. Both have shown that ³H-PCP can bind saturably to tissues other than the brain. Vincent *et al.*,¹³ showed that potencies of PCP derivatives vary in the different tissues. Affinities of various PCP analogues for binding sites in the brain parallel their behavioural potencies in mice, while there is no correlation between behavioural activity and drug binding in liver or kidney.

Thus, while the status of these binding studies is not yet clarified, specific quantifiable PCP receptors may exist. Whatever the final outcome of these studies, they have served to focus attention on a drug class whose effects may hold promise in clarifying abnormal brain function in schizophrenia. □

The transcription of eukaryotic genes

from R. A. Flavell

In the past year two systems have been developed where specific transcription of eukaryotic genes can be obtained *in vitro*. Weil *et al.* (*Cell* **18**, 469; 1979) showed that an S100 extract of mammalian cells conferred specificity on the transcription of adenovirus (Ad) 2 DNA by RNA polymerase II. In this case, the Ad 2 specific RNAs were produced with a 5' end coincident with that of the major late Ad 2 pre-mRNA. These RNAs are therefore presumably directed by the Ad 2 late promotor. Likewise, Manley *et al.* (*Proc. natn. Acad. Sci. U.S.A.* in the press) have shown that specific initiation of RNA synthesis from the same promotor occurs in a total cell extract from Hela cells.

In this issue of *Nature* Wasylyk *et al.* describe the *in vitro* transcription of the conalbumin ovalbumin genes and compare this with adenovirus late and early genes using the system of Weil *et al.* They show that the conalbumin gene and the Ad 2 late region is transcribed efficiently in this system and that the 5' ends of the *in vitro* RNAs are the same as the *in vivo* RNAs. The ovalbumin genes and the Ad 2 early 1A 'promoters' are only poorly utilized in this system. It is not clear at this stage if these differences reflect strong and weak promoters as defined in prokaryotic systems.

Sequence comparison of the 5'

extragenic regions of a number of eukaryotic genes has shown that certain DNA regions appear to be conserved in evolution. In particular, a sequence (TATAAA) similar to the Pribnow box of the prokaryotic promotor, is found around position -30 (i.e. 30 nucleotides upstream from the 1st nucleotide of the mRNA) in most eukaryotic genes (Proudfoot *Nature* **279**, 376, 1979). However, some genes appear to lack a demonstrable Hogness box (notably the late regions of SV40 and polyoma). It is of great interest to determine whether these conserved DNA sequences play a role in the initiation of transcription.

Presumed promoters can also be tested by *in vitro* site-directed mutagenesis and then the deletion mutant tested for its ability to direct transcription in the assay system of choice. Using this approach, Grosschedl and Birnstiel (*Proc. natn. Acad. Sci. U.S.A.* **77**, 1432; 1980) have analysed the transcription of the sea urchin H2A histone genes after injection of the DNA into the nucleus of *Xenopus laevis* oocytes. They showed that deletion of the regions immediately upstream from the H2A gene, and including the Hogness box, reduces, but does not eliminate

R. A. Flavell is in the National Institute for Medical Research, Mill Hill, London.

- Domino, E. F. *Int. Rev. Neurobiol.* **6**, 303 (1964).
- Luby, E. D., Cohen, B. D., Rosenbaum, G., Gottlieb, J. J. & Kelly, R. A. *M.A. Arch. Neurol. Psychiat.* **81**, 363 (1959).
- Luisada, P. V. *NIDA Res. Mon.* **21**, 241-253 (USPHS, Washington, DC, 1978).
- Maayani, S., Weinstein, H., Ben-svi, N., Cohen, S. & Sokolovsky, M. *Biochem. Pharmacol.* **23**, 1263 (1974).
- Melbach, R. C., Glick, S. D., Cox, R. & Maayani, S. *Nature* **282**, 625 (1979).
- Tsai, M. C., Aronstam, R. S., Eldefrawi, M. E., Eldefrawi, A. T. & Albuquerque, E. X. *Fed. Proc.* **38**, 274 (1979).
- Mittag, T. W. & Gross, S. P. *Int. Soc. Neurochem. Abs.* p.486 (1979).
- Albuquerque, E. X., Adler, M., Spivak, C. E. & Aguayo, L. *Ann. N. Y. Acad. Sci.* 1980, in the press.
- Zukin, S. R. & Zukin, R. S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6372 (1979).
- Vincent, J. P., Kartalovski, B., Geneste, P., Kemenka, J. M. & Lazdunski, M. *Proc. natn. Acad. Sci. U.S.A.*, **76**, 4678 (1979).
- Maayani, S. & Weinstein, H. *Life Science* in the press.
- Snyder, S. H., Pasternak, G. W. & Pert, C. B. in *Handbook of Psychopharmacology 5* (eds Iversen, L. L., Iversen, S. D. & Snyder, S. H.) 329-360 (Plenum, New York, 1975).
- Vincent, J. P., Vignon, J., Kartalovski, B. & Lazdunski, M. in *PCP: Historical and Current Perspectives* (ed. Domino, E. F.) (NPP Publications, Ann Arbor, in the press).

transcription of the H2A gene. Interestingly, this deletion appears to cause the synthesis of RNAs with heterogeneous 5' ends located downstream from the normal 5' end.

A number of observations in viral systems point to the same type of phenomenon. Bending and Folk (*J. Virol.* 32, 530; 1980; *Cell* in the press) have isolated viable deletion mutants of polyoma where both Hogness box and cap region are deleted. Likewise Benoist and Chambon (*Proc. natn. Acad. Sci. U.S.A.* in the press) have constructed the equivalent mutants in SV40, which are still able to express the early region. A large deletion removing sequences far upstream inactivates this region. Finally, Gluzman, Sambrook and Frisque (*Proc. natn. Acad. Sci. U.S.A.* in the press) have shown that a large deletion of about 200 nucleotides which eliminates the Hogness box and the upstream sequences apparently inactivates the SV40 transcription unit. It seems, therefore, that loss of the Hogness box causes the production of RNAs with heterogeneous 5' ends when transcription is assayed *in vivo*, and at least in viral systems still greater deletions cause the elimination of transcription.

Clearly *in vitro* transcription assay systems offer another convenient and rapid means of mapping promoter sequences. Wasylyk *et al.* report an initial search in this direction. They show that deleting the DNA sequences upstream from position -44 has no detectable effect on the *in vitro* transcription of the conalbumin gene. However, deletion of the additional DNA sequences from this position to -8 eliminates transcription entirely.

It now seems clear therefore that at least some of the sequences required for the transcription of structural genes by RNA polymerase II are localized in the DNA immediately flanking the gene on the 5' side. This differs from the results obtained with RNA polymerase III based systems where these sequences can be deleted without affecting transcription (Sakonju *et al. Cell* 19, 13; 1980; Bogenhagen *et al. Cell* 19, 27; 1980; Kressmann *et al. Nucl. Acids. Res.* 7, 1749; 1979; Thimmappaya *et al. Cell* 38, 947; 1979).

It seems likely that the Hogness box forms part of the 'promotor' yet proof for this must await specific alteration of this region by fine structure deletions and point mutations. However, *in vivo* results point to a second DNA region, localized further upstream, that is implicated in transcription initiation. One obvious difference between the *in vivo* and *in vitro* data is that *in vivo* the DNA is packaged in some form of chromatin whereas this does not appear to be so in the *in vitro* systems, and perhaps this is involved in the functioning of this second 'region'. One thing, at any rate, is clear — the 5' ends of every gene under the sun are in for a beating with mutagens and nucleases this year in the quest for the eukaryotic promoter. □



100 years

Several papers have stated that an official commission will be appointed in France to witness the crossing of the British Channel by a balloon travelling from France to England (weather permitting). The fact is that the experiment is to be made from Boulogne by M. Jarvis, with his own balloon and at his own risk. But the port authorities have agreed to send M. Jarvis such information as will enable him to select for starting a time when the wind is blowing with some sufficient prospect of reaching England. M. Jarvis will keep watch from June 1 to 20. A steamer will follow as far as possible the hardy aeronaut on his adventurous trip.

The Emperor of Russia has conferred the

Grand Cross of the Order of Stanislaus upon Dr. Hermann Obst, the director of the Ethnographical Museum of Leipzig.

In a series of papers on the northern part of the continent contributed to an Australian paper the writer mentions a curious feature of the creeks and lagoons in the north of Queensland. This is what is called 'floating grass.' It is a tall aquatic grass, which while growing in the mud when within reach, is quite independent in that respect, and extends its creeping stems into the deepest water; and by the interweaving of these, and of the roots emitted from every joint, makes a dense mat of verdure, which, at first sight, seems to have its origin on solid ground. It is however quite possible to walk on it without risk of entanglement. The method is to keep going, lifting the feet well, and with the body in as flat a position as possible. Horse and cattle are fond of this grass, and it is said that the masses of it are sometimes so dense, although with twenty feet of water underneath, that horses have been known to cross on them.

From *Nature* 22, 3 June, 110 & 111, 1880.

Asteroid Hektor

from Jonathan Gradie

THE asteroids are more than just the 'minor planets' of our Solar System. Their small size belies their importance to our understanding of the formation of all the planets. Today we see the asteroids not as the vestige of a disrupted planet, as was once thought, but as the remains of the process which formed the planets. They are not only a window back in time to the beginning of our solar system, but are also a record of the events as they happened in time and space.

The past decade has shown an explosion of knowledge about asteroids. Physical studies have shown the existence of at least two major types of asteroids: the low albedo ($p_v \sim 0.04$), spectrally neutral 'C' objects, and the higher albedo ($p_v \sim 0.13$), spectrally reddened 'S' objects. The 'C' objects are probably similar to if not identical to the carbonaceous chondrite meteorites; the 'S' objects appear to closely match the stony-iron meteorites. The asteroid Vesta has been found to be identical to if not the source of the basaltic achondrites.

One fascinating aspect of recent theoretical work is the study of the collisional evolution of the asteroids. Collisions appear to have dominated the history of the asteroids; collisions can range from minor erosive cratering impacts to major catastrophic events which result in the complete disruption of the body and the formation of a Hirayama family. Depending upon the circumstances of the collision, the final product can take on a most curious form, at least according to Hartmann and Cruikshank (*Science* 207, 976; 1979). They hypothesize that under very special circumstances, some collisions

may result in the accretion of a new and larger asteroid. They cite the Trojan 624 Hektor as a possible example.

Hektor is a strange object. It orbits with the proceeding Trojan cloud at one of the Lagrangian points of Jupiter. The large amplitude (1 mag) lightcurve was originally interpreted as evidence that Hektor was a very elongated, cigar-shaped object ($b/a \sim 1:3$), probably a splinter from a larger asteroid destroyed by a catastrophic collision. Thermal radiometry identified Hektor as a comparatively large asteroid (long axis ~ 300 km) with a very low albedo ($p_v \sim 0.02$). As Hartmann and Cruikshank have pointed out, the combination of the 1:3 elongation and the large size is unusual among asteroids and somewhat puzzling as the other Trojans in the proceeding cloud do not appear to be splinter-like fragments of a much larger parent body destroyed by a catastrophic collision. It appears that the lightcurves may have been interpreted incorrectly, that perhaps the large amplitude lightcurve was due to albedo effects, i.e., one side light, the other dark, instead of shape effects. If the lightcurve is caused mainly by albedo variations this would remove the problem caused by the combination of elongated shape and large size. Hartmann and Cruikshank were able to prove with simultaneous observations in reflected light and emitted thermal radiation that Hektor is indeed highly elongated and that the unusual shape accounts for most of the light variation.

How did Hektor become so elongated?

Jonathan Gradie is in the Center for Radiophysics and Space Research, Cornell University.

The authors hypothesize that instead of a collision fragment, Hektor may be a compound asteroid, that is, the result of the coalescence of two Trojans of comparable size during a very gentle collision. The idea is not as far-fetched as it may seem at first. The average relative velocity of asteroids in the main belt is ~ 5 km/sec, which is more than enough to disrupt two equal-sized bodies, whereas the average relative velocity in the Trojan cloud is only 1-2 km/sec. Some encounters, especially on the low velocity tail of the velocity distribution, will be at a low enough velocity to leave both bodies intact and gravitationally bound.

To account for the discrepancy between the lightcurve amplitude that two spheres in contact would have and the amplitude

observed, Hartmann and Cruikshank propose that the contact zone between the two components may be photometrically brighter in a manner somewhat analogous to rays around fresh lunar craters. Whether or not this material could be detected spectrophotometrically as suggested, remains a problem, but if it is a bright neutrally colored substance, such as ice, it should show clearly.

Low velocity collisions among asteroids in the main belt have been only qualitatively explored. The current suspicion that some asteroids may have satellites or may be binary systems should provide impetus for further study in this area. It may turn out that low velocity collisions are of fundamental importance in the evolution of at least some asteroids.

□

Biology in the 1980s, plus or minus a decade

From Miranda Robertson

GOOD scientists do not speculate a decade ahead of their research. So when the Friedrich Miescher-Institut invited seven good scientists to celebrate its tenth birthday by talking on biology in the 1980's it might have expected to be reminded (by C. Weissmann), that in 1970 no-one would have dreamed that eukaryotic genes were split; (by C. Blakemore) that few could have guessed the importance of peptides in brain function; and (by almost everyone) that new discoveries and conceptual revolutions are by their nature unpredictable. Weissmann put his finger on the difficulty when he quoted the last words of Gertrude Stein, which were: "What is the answer? . . ." followed after a pause by " . . . What is the question?" In science, the next question is always contingent on the answer to the last, and anyone who can see more than one or two questions ahead is probably making up stories, not doing research. Consequently most of the speakers opted to discuss the work of the past decade (or two) and raise some outstanding questions.

K. Illmensee, for example, announced a technical achievement that will make it possible to investigate some hitherto inaccessible processes in mammalian development. He has succeeded in making small clones of mice from the ectoderm and

proximal endoderm of 7-day embryos, by nuclear transplantation into fertilized eggs. Not all embryonic tissues can be used for cloning: the nuclei of trophoblast and distal endoderm are irreversibly differentiated in embryos at that stage. Because of the progressive loss of developmental potential in differentiating nuclei, this offers biologists a new system in which to look for the mechanism of gene inactivation; and it has already enabled Illmensee to investigate the reasons for the abortive parthenogenesis of LT strain mice. LT females spontaneously produce parthenogenetic embryos which sometimes implant but invariably die soon after. By transplanting the nuclei from such embryos into fertilized eggs of another strain, Illmensee has been able to produce adult mice originating from a parthenogenetic nucleus. Since the cytoplasm of LT eggs is perfectly capable of sustaining the development of non-parthenogenetic embryos, this result is rather remarkable and implies either that fertilization provides or triggers an essential factor in the cytoplasm, or that there is a specific failure in the interaction between parthenogenetic nuclei and LT cytoplasm.

D. von Wettstein's contribution to the futuristic spirit of the occasion was a small crate of 1980s experimental lager brewed from a mutant barley with a block in the biosynthetic pathway to the proanthocyanidins, which precipitate proteins and cause lager to cloud. At present, the mutant barley lacks mildew resistance as well as proanthocyanidin, so the brewer avoids treating the beer for clouding at the expense of treating the barley for fungus: but it should eventually

be possible to breed in the disease resistance genes and have the best of both worlds. Two speakers testified that the mutation has no adverse effect of flavour. Although von Wettstein speculated on more sophisticated operations, such as gene transplantation to accelerate the action of brewer's yeast, advances in food (or drink) production have come in practice largely from conventional genetics. Perhaps the 1980s will see the realization in this field of the practical potential of genetic engineering with recombinant DNA.

Certainly Weissmann and his collaborators have already taken an important step towards genetically engineered drug production with the cloning of an interferon gene — or, as it now turns out, two interferon genes. Three different kinds of interferon can be distinguished on the basis of their cellular origin and activity: immune, or γ interferon, fibroblast, or θ interferon, and leukocyte, or σ interferon. Sequencing¹ of the cloned DNA for leukocyte interferon now shows that there are at least three genes for leukocyte interferon alone. The nucleotide sequences of the two genes cloned by Weissmann and his colleagues are distinctly different from one another, and neither corresponds to the amino acid sequence reported by Zoon *et al.*² for leukocyte interferon.

The nucleotide sequence of the fibroblast interferon gene sequenced by Taniguchi *et al.*³, on the other hand, does seem to correspond to the fibroblast interferon amino acid sequence⁴. What the functional significance of the different leukocyte interferons may be it is not possible to guess, but the question is now wide open to investigation.

The discovery of unsuspected genes (pro-opiocortin is another example) and mechanisms (RNA splicing) was no doubt what S. Brenner had in mind when he remarked that progress in science depends on new techniques, new discoveries and new ideas, probably in that order. After a decade of research on the development of the nematode nervous system, he still does not know how it is genetically programmed — possibly because, as J. D. Watson is said to have commented, the work was twenty years ahead of its time. Brenner's own reflection on the project is that the fundamental mystery of metazoan evolution and ontogeny probably cannot be solved by looking at mutations, such as those that he and others have investigated, that have obvious deleterious consequences for all mutant animals. Since each viable species is built from an earlier viable species, the informative mutations may be those with poor penetrance, or barely discernible phenotype, which could have persisted without jeopardizing survival while evolution took its leisurely course.

Miranda Robertson is an associate editor of *Nature*.

*A symposium on Biology in the 1980s was held by the Friedrich Miescher Institut in Basle on March 20-21. The speakers were Colin Blakemore, Chairman of the Department of Physiology, Oxford; Sydney Brenner, Director of the MRC Laboratory of Molecular Biology, Cambridge; Karl Illmensee, Professor of Biology at the University of Geneva; Niels Jerne, Director of the Basel Institute for Immunology, Basel; George Klein, Director of the Institute of Tumor Biology, Karolinska Institute, Stockholm; Michael Soker, former Director of the Imperial Cancer Research Fund and President of Clare Hall, Cambridge; Lewis Thomas, President of the Memorial Sloan-Kettering Cancer Centre, New York; Charles Weissmann, Director of the Institute for Molecular Biology, Zürich; Dieter von Wettstein, Director of the Department of Physiology, Carlsberg Laboratory, Copenhagen.

Such mutations are, of course, extremely difficult to investigate, but techniques for exploring them, at least in structural genes, do exist in what Weissmann chooses to call reverse genetics. The principle is, instead of selecting and manipulating phenotypes to explore the genotype, to manipulate the genotype directly and observe the effect on the phenotype. In practice, it means making discrete and specific mutations or deletions in specific regions of the DNA. By these means Weissmann has, for example, shown through deletions that the "Hogness box" upstream of the rabbit beta globin gene is necessary for the correct initiation of beta globin transcription in mouse cells. It is in principle possible to use reverse genetics to explore the phenotypic consequences, if any, of small mutations in any gene and its regulatory elements. But while that would clarify the "path from gene to phene" (to borrow Hadorn's phrase from the title of Weissmann's talk), it hardly approaches the path from genotype to phenotype which is Brenner's preoccupation.

G. Klein opened up an interesting avenue for exploring the relationship between genotype and neoplastic phenotype. He and his colleagues have shown that trisomy of chromosome 15 is associated with mouse T cell leukaemias induced by widely diverse agents, but not with non-T cell leukaemias. Klein suggests that chromosome 15 may contain genes coding for the differentiated functions of T cells, as well as for the proliferative potential of stem cells. The extra copy of chromosome 15 remains in the undifferentiated stem cell state at

division and enables the leukaemic cell to express both. Another possibility is that the two chromosomes are both in the same state and the neoplastic phenotype is a gene dosage effect. It should be possible to distinguish experimentally between these alternatives and perhaps at last shed some light on the mechanisms underlying neoplastic transformation in this and other leukaemias associated with specific abnormal karyotypes.

Two of the principal outstanding questions for the 1980s are those of the control of embryonic development, and the mechanism of plasticity in the nervous system (which is to say, learning). The answer to the first depends on techniques for identifying morphogenetic substances; and the answer to the second, at least in Blakemore's view, depends on techniques for measuring small changes in synaptic resistance. In neither field is there, or has there ever been any dearth of ideas, so perhaps Brenner is right to put technology first. Certainly recombinant DNA technology provided the means to solve the riddle of immunoglobulin diversity where his own elegant ideas and those of others on their own had failed. And yet there are cases in which the technology is trailing the ideas. Blakemore pointed out that all that the measurement of neuronal metabolic activity by 2-deoxyglucose has done so far is to confirm what was already known from more fragmentary and indirect data. The same applies to the application of recombinant DNA technology to the elucidation of the mating-type switch in baker's yeast⁵.

All that advanced technology can do is to provide the tools with which to answer the questions raised by men and women of ideas; and it is perhaps just as well in these hard times that institutions such as the Friedrich-Miescher Institute exist to shelter them. □

this may be a clue to the physical processes involved in the development of a star from a cloud of dust and gas.

Some idea of distributions of density, velocity, magnetic field and general level of excitation in molecular clouds can be obtained from absorption and emission data. However, as soon as one attempts to go beyond rather general statements, it becomes apparent that we have inadequate knowledge of the structure and properties of many molecules, even of the simplest and commonest. For example there is no data that would allow us to calculate possible processes by which populations of hydroxyl might be inverted. A great deal of laboratory work is required to match the astrophysical observations and some of the issues that are raised are of considerable interest for molecular physics.

A large and awkward gap in our knowledge of interstellar molecules is of the processes by which they might be formed. We know that they are destroyed by photo dissociation and the rates are sometime known; it is clear that molecules are present in dense clouds because there they are shielded from ultra-violet radiation. But the rates at which they are formed are for the most part speculative. The processes of formation are also of interest because laboratory experiments show that some molecules are formed by chemical reactions in excited states from which they can radiate stimulated emission. Molecules, it seems, may be formed from matter absorbed on the surfaces of solid grains, or they may be formed in the gas phase when the participation of ionised species is essential to obtain a sufficiently large cross section. In recent years, calculations¹ and experiment² have provided a rather firm basis on which to construct models of the interstellar gas in which reactions between positive ions and neutral atoms are involved and have led to a good understanding of the formation of carbon hydrogen molecules in particular³. There is evidence⁴ that reactions of neutral atoms with molecular ions may be important and the results of a collaboration between two of the principle experimental groups in this field (those at NOAA Boulder, Colorado, and at Birmingham University) have recently been published⁵. A newly developed technique was used to enable neutral atoms to be injected into a flowing beam of molecular ions. Twenty four reactions of various molecular ions, such as

1. Mantei, N. *et al. Gene* (in the press).
2. Zoon, K. C. *et al. Science* **207**, 527 (1980).
3. Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. & Maramatsu, M. *Gene* (in the press).
4. Knight, E., Hunkapillar, M. W., Korant, B. D., Hardy, W. F. & Hood, L. E. *Science* **207**, 525 (1980).
5. Leupold, U. *Nature* **263**, 811 (1980).

Formation of interstellar molecules

from A. H. Cook

SINCE the first observations of microwave radiation from atomic hydrogen some thirty years ago many molecules have been detected in interstellar space. Using measurements of absorption, spontaneous emission and stimulated emission, results of general importance in astrophysics have been obtained.

Carbon monoxide is widespread in the galaxy and because it is excited above the ground state predominantly by collision with molecular hydrogen, its radiation can be used

to estimate the density of molecular hydrogen. This has led to the important conclusion that the distribution of molecular hydrogen follows the spiral arms as traced out by stars and atomic hydrogen, and that the amount of molecular hydrogen in the galaxy is about the same as that of atomic hydrogen. Most molecules are not widespread but seem to occur in dense clouds of dust and gas associated with very young stars, their presence thus seems to be related to the processes by which stars are formed. It is found that the populations of the different states of molecules in such regions almost never follow the Boltzmann distribution, they are not in thermodynamic equilibrium, and

1. Black, J. H. and Dalgarno, A. *Astrophys. J. Suppl.* **34**, 405 (1977).
2. Sinnott, G. *NBS Spec. Pub.* **381**, Washington: Govt. Printing Office (1973); Ferguson, E. E. *At Data Nucl. Data Tables* **12**, 159 (1973); Huntress, W. T. *Astrophys. J. Suppl.* **33**, 495 (1977); Albritton, D. L. *At Data Nucl. Data Tables* **22**, 1 (1978).
3. Smith, D. & Adams, N. G. *Astrophys. J.* **217**, 741 (1977); Smith, D. & Adams, N. G. *Astrophys. J. Lett.* **220**, L87 (1978).
4. Fehsenfeld, F. C. & Ferguson, E. E. *J. Chem. Phys.* **56**, 3066 (1972); Fehsenfeld, F. C. *Astrophys. J.* **209**, 638 (1976); Karpus, Z., Anicich, V. & Huntress, W. T. *J. Chem. Phys.* **70**, 2877 (1979).
5. Viggians, A. A. *et al. Astrophys. J.* **236**, 492 (1980).

A. H. Cook is Professor of Physics at the University of Cambridge.

CH^+ , CH_2^+ , C_2H_2^+ , CH_3^+ , H_2O^+ and others, with N_2O and NO were studied. The rates are often relatively high, and many of the reactions are clearly likely to be important in interstellar chemistry. The reaction schemes indicate once again the

importance of the HCO^+ molecular ion and of reactions with H_2 .

This work not only provides much useful data but also demonstrates the importance of laboratory studies in the interpretation of interstellar chemistry and physics. □

	I/E	$\iota-\epsilon$
Harwood & Malin	0.12	8°
Yukutake & Cain	0.32	105°
Ducruix <i>et al.</i>	0.17	44°

Geomagnetic secular variation and the conductivity of the lower mantle

from David R. Barraclough

THE slow changes in the Earth's magnetic field with time provide one of the very few means to investigate the dynamics of the Earth's fluid core and the electrical conductivity of the lower mantle. Observations of the geomagnetic field changes — the secular variation — have been made ever since the seventeenth century because of their practical importance for those using magnetic charts. Nowadays, a network of 200 permanent magnetic observatories provides very accurate data free from spurious effects associated with changes of observing site.

The task of forecasting the secular variation for several years ahead is complicated by any sudden changes which may occur. Such a change appears to have taken place in about 1970. It was first noticed in the data from European observatories (Courillot *et al.* *Comptes rendus* **D287**, 1095; 1978) but it has since been shown to have had world-wide effects (Ducruix *et al.*, *Geophys. J.R. astr. Soc.* **61**, 73; 1980; Malin *et al.* *Ebro Obs. 75th Anniversary Volume* in the press). The phenomenon manifested itself as a sudden change in the secular acceleration, the second time-derivative of the main geomagnetic field (Barraclough & Malin, *Geophys. J.R. astr. Soc.* **58**, 785; 1979). Ducruix *et al.* found a positive change in the east component of the secular acceleration over Europe and central Russia and a negative change in this component over eastern Asia and north-western North America. Malin *et al.* examined the changes in the secular acceleration of the magnetic declination, the horizontal intensity and the vertical component. Their results for the declination are in agreement with the French results and they also find regional patterns in the other elements. Interestingly, all three elements suffered a sudden decrease in the secular acceleration over North America. Using a homogeneous set of observatory annual means covering the period from 1961 to 1978, Malin *et al.* have produced a spherical harmonic model of the phenomenon which clearly shows the details of its global distribution. There is general agreement that these sudden changes occurred on a time-scale short in

comparison with that of the secular variation itself: certainly less than 4 years.

Most geomagnetic parameters vary with the sunspot cycle, which has a period of approximately 11 years. Observatory annual mean values show such a sunspot cycle variation (RV) which, although small in amplitude, can have a significant effect on secular variation estimates derived from the annual means. The source of RV is believed to lie above the Earth's surface, within the magnetosphere. Because the Earth has a finite electrical conductivity, these external magnetic field variations will induce electric currents within the Earth, together with associated magnetic fields. Thus, what is observed at the Earth's surface is a combination of the external inducing field and the internal induced field. It is possible, however, by considering the horizontal components and the vertical component of the variation field separately, to separate the internal and external parts. The ratio of the amplitudes of the two parts and their phase difference can then be used to investigate the conductivity of the regions of the Earth where the induced currents flow — the lower mantle in the case of RV. Three studies of RV have recently been published. Ducruix *et al.* (*op. cit.*) used the data from the 10 European observatories which provided the evidence for the sudden change in secular acceleration. The data spanned the interval 1947 to 1977. Yukutake and Cain (*J. Geomagn. Geoelect. Kyoto* **31**, 509; 1979) based their main conclusions on data from 21 observatories, globally distributed, and covering the period from 1900 to 1973. Harwood and Malin (*Geophys. J.R. astr. Soc.* **50**, 605, 1977) used all available data from 81 observatories with a world-wide distribution. Their earliest data were for 1842 and the most recent for 1974. The table below gives the amplitude ratios (I/E) and the phase differences ($\iota-\epsilon$) which result from these studies. The estimated uncertainties are approximately 0.1 for the ratios and 30° for the phase differences. Ducruix *et al.* have shown that the results

of Yukutake and Cain are not compatible with simple induction models. Considering the uncertainties, the results of the other two studies are in general agreement.

Ducruix *et al.* have used their values of I/E and $\iota-\epsilon$ to test a series of four-layer models of mantle conductivity. The results indicate that the maximum value of the conductivity (which occurs at the bottom of the mantle) is approximately $100\Omega^{-1}\text{m}^{-1}$. The results of Harwood and Malin, which are based on a much more extensive collection of data, are also consistent with a maximum value of $100\Omega^{-1}\text{m}^{-1}$.

Runcorn (*Trans. Am. geophys. Un.* **36**, 191; 1955) showed that, for a change of the core field with a duration of 4 years to be detectable at the Earth's surface, the conductivity of the lower region of the mantle cannot exceed $100\Omega^{-1}\text{m}^{-1}$. Since the sudden change of secular acceleration about 1970 lasted for less than 4 years we are lead to the same upper limit for the conductivity as that given by the RV studies.

These two pieces of evidence thus cast considerable doubt on the higher estimates of lower mantle conductivity such as the figure of $10^4\Omega^{-1}\text{m}^{-1}$ proposed by Stacey *et al.* (*Trans. Am. geophys. Un.* **59**, 1027; 1978) and that of $10^5\Omega^{-1}\text{m}^{-1}$ favoured by Alldredge (*J. geophys. Res.* **82**, 5427; 1977).

Much work remains to be done in extending the studies of RV and the sudden changes in secular acceleration and in applying the results to determining more accurately the conductivity distribution in the lower mantle. The primary requirement is a good set of data, well distributed over the Earth's surface. The existing network of permanent magnetic observatories, which provide the most valuable data for such studies, has a very patchy distribution, very dense in Europe but very sparse throughout most of the Southern Hemisphere. Satellite surveys, such as that of the intensity and direction of the geomagnetic field currently being performed by Magsat, can only help if they are repeated at frequent intervals and even then they present their own set of problems. As far as the practical uses of the secular variation in the production of magnetic charts are concerned, we probably have sufficient knowledge of RV to attempt to correct secular variation estimates for its effects. The problem with the sudden changes is that they are, at present, completely unpredictable. Here, frequently repeated satellite surveys might help in detecting such changes as soon as possible after their occurrence. □

David R. Barraclough is in the Geomagnetism Unit, Institute of Geological Sciences, Edinburgh.

REVIEW ARTICLE

The Western English Channel— an inconstant ecosystem?

A. J. Southward

Marine Biological Association, Plymouth, Devon PL1 2PB, UK

Changes in the chemistry and biology of the English Channel waters off Plymouth in the 1930s were a talking point for marine scientists 30 and 40 yr ago. The reversal of many of the changes, beginning in 1965 and continuing to 1979, may cause equal controversy about the stability of the ecosystem and the factors causing its fluctuation

THE western part of the English Channel off Plymouth has been studied for more than 75 yr, and continuous observations, interrupted only in 1940–45, are available from 1924. Between 1930 and 1936 there was a sequence of changes in abundance and species of zooplankton and pelagic fish, accompanied by a fall in the winter maximum of inorganic nutrients¹. After 1964 these changes began to reverse, and within a few years it appeared that a cyclic change had taken place, coinciding with fluctuation in climate of the Northern Hemisphere². More recent observations suggest the cycle has returned to a state close to that of the 1920s, providing an opportune moment to review the various factors, including water movements, sea temperature and nutrient chemistry, that have been suggested as causes of the cycle.

The changes

The complex series of events in the Western English Channel since the 1920s has been called the 'Russell Cycle' after its principal investigator³; some examples are shown in Fig. 1, and the initial phase of the cycle is mentioned in many textbooks⁴. Basically, a plankton community characterized by the chaetognath *Sagitta elegans*, was replaced over a period of about 5 yr by a community characterized by the more neritic species, *Sagitta setosa*⁵; there was a fall in the winter maximum of dissolved inorganic phosphate (Fig. 1c), one of the nutrients used by phytoplankton each spring; the standing crop of zooplankton and the numbers of planktonic stages of demersal fishes (Fig. 1c) decreased by an order of magnitude or more⁶; there were decreases in certain species of the benthos and replacement of some cold-water demersal fishes by warmer-water species⁸; the final event was the dramatic failure of the herring fishery off Plymouth in 1936 (ref. 9). At that time, the message of these changes seemed clear: a reduced flow of water into the Channel from the west led to a decline in nutrients; a lower level of nutrients would limit phytoplankton production¹⁰; a lower primary production would mean lower crops of copepods and other herbivores; fewer young fish would survive, and adult plankton-feeding fish would migrate elsewhere to find food; reduction in the zooplankton and pelagic fish would lead to decline of other fishes and the benthos. Research programmes were, therefore, directed to a study of natural events or processes likely to

control upwelling of 'nutrient-rich' water from the ocean and increased flow through the Channel^{11,12}.

After the major changes between 1930 and 1936 there was a long period, to 1961, without obvious trends; the inorganic phosphate fluctuated about a lower level, *Sagitta setosa* was the dominant chaetognath, and abundance of zooplankton and post-larval demersal fish remained low^{13–15}. However, from the presence of large numbers of pilchard eggs in the plankton samples (Fig. 1b) and from echo-sounder surveys it became obvious that the numbers of adult pilchard must have greatly increased after 1935, and that this fish had replaced herring as the dominant pelagic fish^{16,17}. A new hypothesis was put forward, that herring had failed in competition with pilchard¹⁷; the presence of large numbers of overwintering young pilchard might be sufficient to account for the lower maxima of inorganic phosphate, since they would contain, locked up in their flesh and bone, phosphorus and nitrogen that would otherwise have returned to the water each winter by death and decay of more ephemeral plankton organisms.

Search for an external cause of the increased population of pilchard was made easier by the longer series of environmental records becoming available. It was found that the waters of the Channel had warmed up between 1900 and 1950, during the general amelioration of climate of the Northern Hemisphere¹⁸, and much of the rise had occurred in the 1920s^{19,20}. Evidence had also accumulated for increased occurrences of warm-water fishes^{8,21} and increases in the warm-water elements of the fauna of rocky sea-shores²². It was, therefore, possible to put forward a more general hypothesis to cover all the changes observed up to 1961⁸; that as a result of the climatic change there had been northward extensions of the boundaries of distribution, not only of boreal communities into the Arctic²³ but of warm temperate communities into the boreal regions. The *S. elegans* community is predominantly of cold-water origin, and herring are close to their southern limits at the entrance to the Channel. Pilchard, and many of the plankton species associated with the *S. setosa* community off Plymouth, are of warm-water origin. A shift in distribution of this nature, accompanied by slight alteration in the water circulation pattern in the Western Channel as a result of the climatic change (increased southerly influence) was regarded as sufficient to account for most of the changes in the ecosystem⁸.

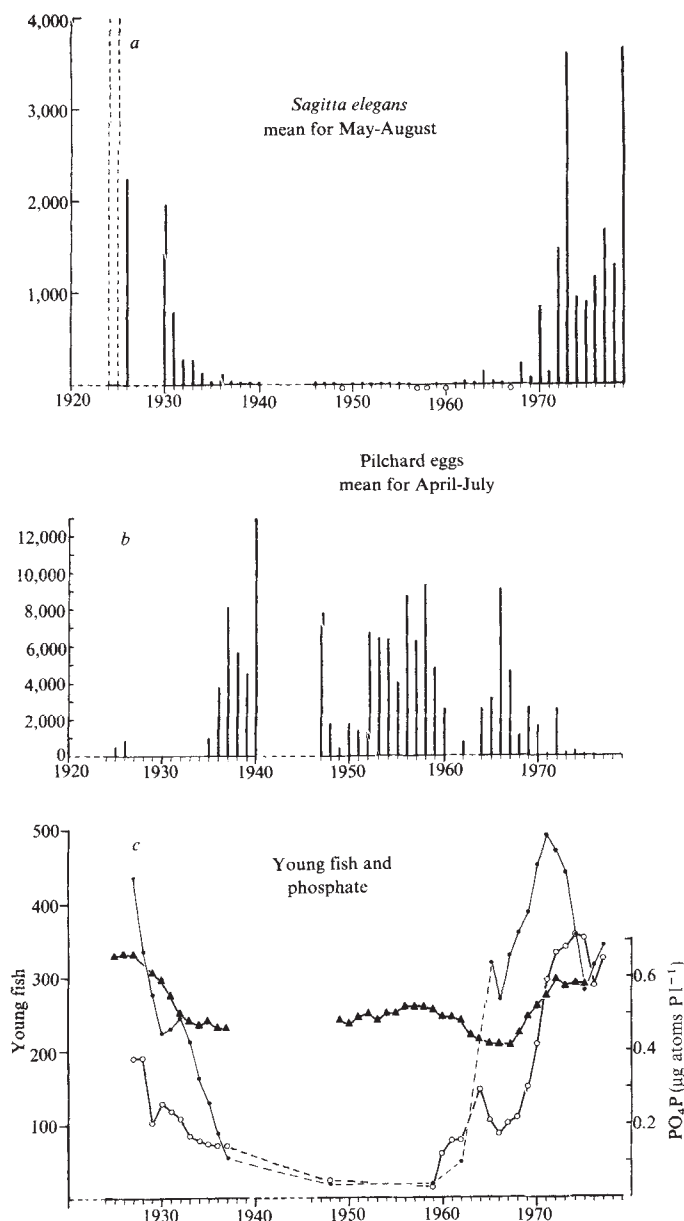


Fig. 1 Examples of some of the biological changes off Plymouth, based on weekly samples with the 2 m net at station L5 (2 miles east or 2 miles west of the Eddystone reef). The standard oblique haul filters about 5,000 m³ of water⁶⁹. As indicated by the broken line for the ordinates, sampling was not possible in certain years; the broken columns or broken trend lines show years when sampling was incomplete. *a*, Numbers of the chaetognath *Sagitta elegans* Verrill, expressed as the average per haul for the summer months (May to August inclusive) when the offshore water is stratified. *b*, Numbers of eggs of pilchard (*Sardina pilchardus* (Walbaum)) expressed as the average per haul during the spring/summer spawning season from April to July inclusive. The number of eggs is assumed to be an index to the numbers of mature adults¹⁶. *c*, Comparison of smoothed values (5-yr running means) of inorganic phosphate (▲) at station E1 (50°02'N, 4°22'W), with 5 yr running means of post-larval teleosts (excluding clupeids) in the 2 m net samples at L5. The young fish are divided into spring-spawners (●), taken as the sum of the monthly means for March to June, and summer-spawners (○), taken as the sum of the monthly means for July to September. Phosphate is expressed as the 'winter maximum' of 'reactive' inorganic phosphate in µg atoms P l⁻¹. The winter maximum can be found variously from December to early March, and is allocated here to the spring of the following year if found in December.

The pilchard seemed to be associated with a more 'efficient' ecosystem²⁴, with a shorter food chain²⁵, smaller standing crops of zooplankton, and less 'wastage' to the benthos, and the fall in inorganic nutrients was seen as an index to the changed ecosystem rather than as the primary cause.

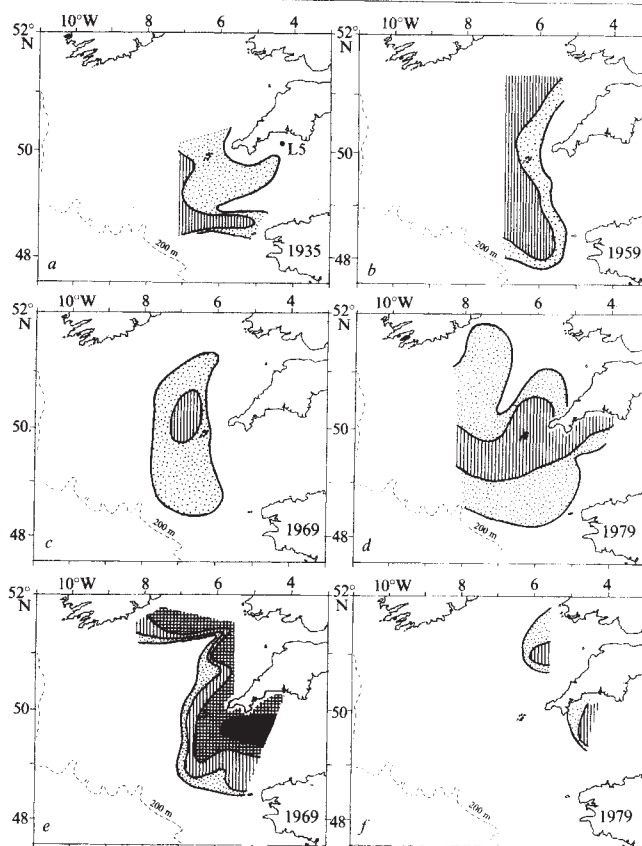
With the formulation of this hypothesis in 1963 we had reached the point where selection between the different theories could only come from new evidence, either by further changes or by a reversal or previous changes⁸. As if in answer to an ecologist's prayer the system did in fact go into reverse in the second half of the 1960s, and investigation was much stimulated. The numbers of post-larvae of demersal fishes increased from 1965, and by 1970 numbers of copepods had reached levels comparable with those of 1930². The period of maximum spawning of pilchard shifted from early summer to autumn, and then both spawnings began to decline²⁶; by 1975 there were virtually no pilchard eggs, and presumably no adult pilchard, off Plymouth in the first half of the year. However, herring did not return in large numbers, and mackerel became the dominant pelagic fish²⁷.

There was a slowing down of the rate of reversal of the cycle in the 1970s, with annual fluctuations in the ecosystem similar to those detected from 1931 to 1935 in the first phase of the cycle^{28,29}. Each summer, from 1972 to 1978, there was a period with a plankton community characterized by *Sagitta elegans* and other northwestern plankton indicators^{15,30} but the total dominance of *S. elegans* reported before 1930 was never achieved. However, early in 1979 changes became more noticeable. The first sign was the occurrence on poor-cod (*Trisopterus minutus*) of a parasite normally found on the gills of Norway-pout (*T. esmarkii*)³¹ a northern distributed gadoid fish only once before recorded from the Western Channel³². It was soon confirmed that *T. esmarkii* itself had moved into the district, together with larger numbers of blue-whiting (*Micromesistius poutassou*), another small gadoid of cold-water oceanic distribution³³. The plankton changed from February, and *S. elegans* became the dominant chaetognath, *S. setosa* being scarce or absent to the end of August. The typical associate species of pure 'elegans' type, the trachymedusan *Aglantha digitalis* was present from April, and in July this species underwent a very big increase in abundance, with a maximum of 15 m⁻³ in August and average numbers equal to those reported in July and August 1930 (ref. 29). There was a temporary switch in the autumn of 1979 to a more typical warm-water or southwestern plankton community, with the trachymedusan *Liriope* replacing *Aglantha*, the siphonophore *Muggiaea atlantica* replacing *Nanomia* species; and the copepod *Euchaeta hebes*, some pilchard eggs and, of course, *S. setosa*, were present. A transition of this sort has occurred during the autumn of many years, marking a period of increased flow round Ushant³⁴ and coinciding with the breakdown of the thermocline off Plymouth, but in 1979 it was brief, and the dominance of the *elegans* community was restored in November and has continued up to the time of writing.

Figure 2*a-d* compares the distribution of *Aglantha* in the Western Channel and Celtic Sea in July of 1979, with the results of similar surveys made in previous decades during the period of dominance of *S. setosa* and pilchard off Plymouth, and with the results of a survey made in 1935, towards the end of the first period of change of the Russell cycle³⁵. This species made a very big advance eastwards between 1969 and 1979, of the order of 100 nautical miles; and as shown in Fig. 2*e, f*, there was a corresponding withdrawal of the neritic chaetognath, *S. setosa*, from the entrance to the Channel. These particular changes took place well after the increase in demersal young fish and zooplankton at the Plymouth stations.

Except for return of herring, the ecosystem of the Western Channel off Plymouth has evidently returned to a stage comparable with that of the 1920s. This brief recapitulation of

Fig. 2 *a-d*, Distribution of the trachymedusan *Aglantha digitalis* O. F. Müller in the Western Channel and Celtic Sea in July, as sampled with a 1-m net (1935, 1969, 1979) or the Gulf III sampler (1959). The contours correspond to 100 and 1,000 individuals per 10 min haul of the 1 m net, filtering about 750 m³ of water. *e, f*, Distribution of the chaetognath *Sagitta setosa* J. Müller, in July 1969 and 1979 as sampled with the 1 m net. The contours correspond to 1, 10, 100, 1,000 individuals per haul. The position of the routine sampling station (L5) is shown on *a*.



events over a 55-yr period inevitably omits much detail of the changes; describing the cycle in terms of two contrasting ecosystems (*Sagitta elegans* + herring/mackerel versus *Sagitta setosa* + pilchard) is perhaps an oversimplification, but this is the easiest way to relate the changes to causative factors and discuss the apparent competition between the two systems.

Possible causative factors

Three hypotheses concerning the causes of the change have already been mentioned: (1) nutrient control; (2) competition; (3) local effects of climate change. Other hypotheses to be considered may be called: (4) indirect effects of change in climate³⁶; and (5) natural fluctuations in species equilibrium. None of these is attributed to a single author as all are essentially syntheses of several ideas.

(1) *Nutrient control*. Essentially this hypothesis assumes that many of the changes in the Russell cycle are the result of variations in the flow of water from the west into the Channel¹. In these latitudes *Sagitta elegans* is more of an offshore species ('intermediate' or mixed coastal and oceanic water) than *S. setosa*^{5,15} and a change of community from '*elegans*' to '*setosa*' such as occurred in 1930–35 implies increased coastal influence. The simplest way this could have happened is by reduction of the flow of oceanic water into the Celtic Sea and corresponding reduced flow of mixed oceanic/coastal water through the Channel. The retreat of *S. setosa* shown in Fig. 2*f* illustrates a reduction of coastal influence during the reverse phase of the cycle, and on this hypothesis implies increased flow from the west. The '*elegans*' community has been shown to be associated with increased numbers of the planktonic stages of demersal fish³⁷ and better survival of invertebrate larvae³⁸, but these qualities do not explain all the changes. A critical role is, therefore, allotted to the inorganic nutrients which are assumed to need continuous renewal if primary production is to be maintained in coastal waters³⁹. On this hypothesis the change in the plankton

community is an index to a more fundamental change in hydrography, possibly linked to sinking of cold water in the Arctic, and thus indirectly influenced by climatic change^{11,12,19}. Therefore the greatest changes in zooplankton abundance would be expected to accompany or follow the changes in nutrients. During the first phase of the Russell cycle the major fall in winter phosphate occurred between 1931 and 1939, accompanying the change in the more characteristic species, and preceding the period of maximum decline in zooplankton abundance. However, in 1965–75 the increase in zooplankton abundance, especially of the young stages of spring spawning demersal fish² preceded the rise in phosphate by several years (Fig. 3). Many of the changes in the second phase of the cycle are a mirror image of those in the first phase^{3,30}; in the 1930s *S. elegans* declined first, then young of summer spawning demersal fish, then spring spawning demersal fish, while in the 1960s and 1970s the numbers of spring spawners increased first, then summer spawners, and finally *S. elegans* returned to dominance. Although some aspects of the reversal of the cycle might be expected to take place gradually, depending on the build-up of perennial populations, this is not the order that would be anticipated if water movements and inorganic nutrients were the most important environmental factors governing the cycle. Examination of some details underlines this defect; for example, the apparent correlation between winter phosphate and numbers of summer spawned fish⁴⁰. For the period 1924–38 we have 12 pairs of values of phosphate and young fish, giving a reasonable significant relation ($n=12$, $r=0.77$, $P=0.01$), but for 1964–79 the significance is less ($n=16$, $r=0.59$, $P=0.02$). Taking the whole cycle there is still some significance in the figures ($n=33$, $r=0.49$, $P=0.01$), but at this value for the correlation coefficient the inorganic nutrients cannot be regarded as the overriding cause of the change. The correlation between winter phosphate and numbers of *Sagitta elegans* shows a similar reduction in significance in passing from the first phase of the cycle ($n=11$, $r=0.82$, $P=0.01$) to

the second ($n=16$, $r=0.55$, $P=0.05$). For the numbers of spring spawned young fish there is no relation at all, and some of the other changes are also out of phase with the phosphate values. An additional drawback of the nutrient control theory is that some of the primary production in coastal waters is related to organic nutrients (with which ammonia is classed, as distinct from nitrate)⁴¹. There is much current discussion on the rate of turnover of nutrients in the spring during the phytoplankton outburst and on the proportion of primary production attributable to such recycling as compared with the proportion, the 'new' production, that results from injection of nutrients into the euphotic zone from deeper water, or by runoff from the land and fixation of atmospheric N_2 (refs 42, 43). There are no direct measurements of recycling in the Channel. Using analyses for other regions⁴³, it can be calculated that, of the average annual primary production off Plymouth⁴⁴ (152 g carbon assimilated per m^2 per yr) over 60% might be due to recycling rather than 'new' nutrients. The average difference in the winter maximum of inorganic phosphate between the '*elegans*' period and the '*setosa*' period was 23%. If we assume that all of this represents additional nutrients replenished by increased flow

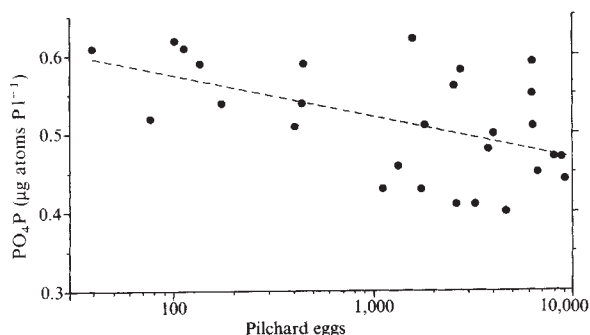


Fig. 3 Regression of winter maximum of inorganic phosphate $\mu\text{g atoms P l}^{-1}$ on $\log_{10}$ numbers of pilchard eggs the previous summer, as average per haul of the 2-m net from April to July. The broken line is a least squares fit.

through the Channel, then the corresponding difference in 'new' production would be only 10% of the annual production. This difference is small compared with the changes of one or two orders of magnitude in the standing crop of zooplankton. A further argument in this direction is provided by recently developed methods for analysis of total dissolved nutrients⁴⁵. Not much was known about the organic fractions during the earlier phase of the Russell cycle but the new data suggest that the totals of N and P undergo much less fluctuation than the inorganic components, and that it is the position of the equilibrium between the organic and inorganic fractions that has changed. This equilibrium may be related to environmental effects or to biological factors or both, but its relative position need not imply any alteration in the primary production of the system⁴¹.

(2) *Competition*. This hypothesis assumes that the major event in the Western Channel was a competitive change of the species of pelagic fish, pilchard becoming increasingly more successful after 1925¹⁷. It is based on data for recruitment to the former Plymouth herring fishery⁴⁶ and the number of eggs of pilchard in the plankton, taken as an index to the adult stock of pilchards^{17, 25}. The increased numbers of young and adult pilchard are assumed to be responsible, through changes in the level and intensity of predation, for the other changes in the ecosystem. From the data available up to 1960 the link between herring and pilchard could be made through the winter phosphate values: there was a negative correlation between log summer pilchard eggs and phosphate the following winter, and a positive correlation between herring

recruitment (year class strength) and winter phosphate a year after hatching. The correlations were with the same value of winter phosphate, indicating a possible direct link between the two fish, irrespective of the exact role allotted to the phosphate¹⁷; in other words, increasing numbers of pilchard were eating up the young herring or their food.

This hypothesis has been extended to cover the effects of climatic change³, by assuming that the degree of synchrony between the spring plankton outburst and the breeding of fish (or other animals) will critically affect the success or otherwise of the brood. Northern species tend to spawn at a fixed time in the spring, and will suffer more from a mismatch with their planktonic food if for example their rate of development is increased by rising temperatures or if phytoplankton begins to develop too early³. Unfortunately the data from the Channel no longer give much support to the idea of direct competition between pilchard and herring. Some missing data have been found for certain years before 1960, some of the phosphate values revised, and the series continued to 1979. The new data (Fig. 3) give an apparently significant correlation between log summer pilchard eggs and inorganic phosphate the winter after, in spite of much scatter, ($n=32$, $r=0.54$, $P=0.01$), but an almost as significant correlation can be shown between log pilchard eggs and phosphate the year before ($n=32$, $r=0.48$, $P=0.01$). Thus any direct effect of pilchard on phosphate (or herring) can be discounted, and we are left with a general similarity in long-term trends, with a tendency for less than $0.5 \mu\text{g atoms of inorganic phosphate-P per litre}$ to be associated with the period when there were more than 100 pilchard eggs per haul in summer.

An alternative version of the competition hypothesis supposes that for some reason the herring declined, allowing the pilchard to move in^{8, 17}. A possible cause of the decline in herring might have been increased fishing pressure, such as we know occurred in the 1920s with the introduction of new technology⁸. In spite of the well known effects of 'density-dependent' factors on year class strength of fish populations, a species which is being heavily fished may be unable to produce enough young stages to ensure their survival through mortalities caused by environmental factors^{47, 48}, especially if the environment itself is changing⁴⁹. However, the subsequent change from pilchard to mackerel cannot be attributed to overfishing and hence other causes of the cycle must be examined.

(3) *Local effects of change in climate*. The climate control hypothesis assumes that geographical distributions of species and communities alter in response to fluctuations in climate. It is argued that rises or falls in sea temperature, translated into north/south shifts of distribution, may be seen as fairly sudden switches of the ecosystem if observations are being made close to faunistic boundaries⁸. For example, herring is a boreal species close to its southern limits at the entrance to the Channel, whereas pilchard is a warm-temperate species close to its northern limit of regular breeding²⁶. The marked rise in temperature in the 1920s is assumed to be the final stimulus that caused the herring to fail and the pilchard to extend its range north⁸, and that there were corresponding shifts in species balance at other trophic levels. The disappearance of *Sagitta elegans* from the Plymouth area is regarded as an example of a retreat of a northern species. However, it was not replaced by the corresponding warm-temperate species *S. friderici*⁵⁰, which appeared only in small numbers during the warmest phase of the cycle in the 1950s and 1960s (ref. 15), but by the more neritic species *S. setosa*. The theory suggests that *S. setosa*, which has a more southern distribution than *S. elegans*, was better able to withstand the wider range of temperatures experienced^{8, 51}. An alternative, but not exclusive explanation was suggested, that the change in climate, especially the greater incidence of southerly and westerly weather⁵¹, may have altered the emphasis of water movements in the Western Channel⁸, bringing in a more prolonged

cyclonic phase from the south and east and reducing the anticyclonic 'spring' phase from the north and west⁵². Such an alteration of circulation pattern could have helped *S. setosa* to become dominant. It was originally implicit in the temperature control hypothesis that much of the decline in the standing crop of zooplankton resulted from increased predation by pilchard, and that the reduced level of winter phosphate was probably related to the increased numbers of pilchard^{8,17}. However, the new data for the latter shown in the previous section indicate an indirect effect, through the general change in ecosystem.

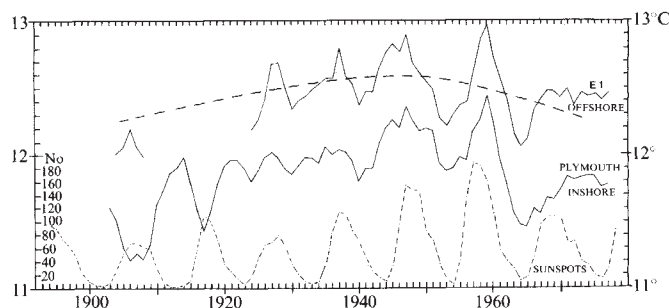


Fig. 4 Comparison of 5-yr running means of annual sea temperature offshore at station E1, 5-yr running means of annual sea temperature in Plymouth Sound, and mean annual (Zurich) sunspot numbers. The broken line is the long term trend, expressed as a polynomial regression fitted to the annual means for station E1. Note the change in phase agreement in inshore temperature and sunspots before 1930; the change in phase agreement between the E1 temperatures and sunspots since 1970; and the good agreement between E1 temperatures and sunspots from 1925 to 1965²⁷.

A recent re-examination of the relationship between trends in sea temperature in the Western Channel and some of the biological changes apparently supported the hypothesis of temperature control²⁷. Correlations could be shown not only with the long-term secular trend, but with shorter term fluctuations of the same frequency as the 11-yr solar cycle. Similar effects of the shorter period cycle as well as longer term trends can be seen in records of sea temperature and plankton abundance for the North Atlantic⁵³⁻⁵⁵. In 1975 it was predicted²⁷ that there would be further gains of northern species in the Channel if the trend towards cooling continued in the Northern Hemisphere⁵⁶, and this has been borne out by events in 1979-80. However, a recent assessment of aspects of the change between 1970 and 1979 suggests a lack of synchrony between the annual mean sea temperature in the Western Channel and the short-period solar cycle (Fig. 4). This seems to be predominantly a result of higher than average temperatures during the first four months of each year from 1972. There is still a degree of correlation between the 11-yr solar cycle, sea temperatures in the latter half of the year, and some of the biological changes, but the apparent phase change during the first half of the year points to advection and weakens the argument for direct effects of temperature. There is also evidence that the current 11-yr sunspot cycle is not behaving as predicted^{57,58}.

(4) *Indirect effects of change in climate.* This hypothesis assumes that the balance between the 'elegans' and 'setosa' communities in the Western Channel depends on the rate of flow of oceanic water from the west and through the Channel into the North Sea³⁵. Herring are assumed, in these latitudes, to be better adapted for survival in the ecosystem of the mixed coastal/oceanic water, pilchard in the more coastal type of plankton community. The change of climate in the Northern Hemisphere is presumed to have produced local changes in the flow of water through the Channel. During the warm period, 1920-60, the extension of the North Atlantic Drift into the high Arctic, and the wider spread of the Gulf Stream

Gyre^{59,60}, may have led to weaker flow in the latitude of the Channel, in spite of the increased frequency of 'westerly' weather at the time⁶¹. With a return of colder conditions, less penetration into the high Arctic, and a tightening of the Gulf Stream Gyre, it could be assumed that there would be greater flow into and through the Channel. Unfortunately, much of the data for flow through the Channel are incomplete or circumstantial. The measurements on the electric power cable across the Straits of Dover are for short periods only⁶², and no consistent trend can be seen. The current meter readings at the Varne Light Vessel⁶³ were discontinued many years ago, and it was never satisfactorily shown if this site was in the main flow or an eddy¹⁵. The circumstantial data from biological observations indicates movement of biota from the Eastern Channel into the southern North Sea after 1970, in harmony with the period of return of *S. elegans* to the Plymouth area^{64,65}.

There are two principal arguments against this concept of a controlling role for climate induced changes in water flow through the Channel. One is that the sequence of events in each of the two phases of the cycle was different; in the second phase the change began in 1965, but the distribution of *Sagitta* and *Aglantha* did not alter substantially until after 1969 (Fig. 2), and *S. elegans* did not regain its former dominance until 1973-79. The second is that there is no evidence of salinity trends (ref. 55 and E. I. Butler, personal communication), such as would be expected if there had been variation in the flow of oceanic water from the west. Thus we are left with an incomplete explanation, and we have to assume some direct effects of temperature, including a shift in fish populations (F. S. Russell, personal communications).

(5) *Natural fluctuations in species equilibrium.* This hypothesis stems from recent observations on primary production in the Western Channel⁴⁴. Although the 10-yr series began only 1-2 yr before the start of the reversal of the Russell cycle, it is clear that there was no dramatic or lasting change in primary productivity off Plymouth as measured by fixation of ¹⁴CO₂. The mean annual production at St E1 was 152 gC per m² per yr, with a standard deviation of ± 29 , and the values for 1964 and 1965 do not differ significantly from later years of the fully changed ecosystem. Thus, as drastic as the change may have been from the economic point of view (in fish populations), it was hardly fundamental at the level of primary production. Note that although variations in pelagic fish populations tend to preoccupy biologists (E. I. Butler, personal communication), these fish account for less than 5% of the annual production. We may postulate that there is normally considerable fluctuation, within the confined level of primary production, of the species dominant at each trophic level. Increase of one species implies reduction of another, or changes in the food chain, so that the total balance of production and utilization is maintained. Such a state may be compared with what the economists among Eurocrats inelegantly term a 'snake in the tunnel'. The change from herring to pilchard and then to mackerel can be seen as a sequence of such 'snakes'. Each species, when it becomes dominant, will tend to stabilize the system for a while, so that the plankton community remains at the optimum structure to support the predator at the top of the ecological pyramid. A process such as this would be akin to that described as 'rectification' in the improved theory of competition³. It can be assumed that there is a tendency for climatic change to favour natural fluctuations more in one direction than the other, for example, rising temperatures might put an end to a period of herring dominance, but on this hypothesis such a shift would not be possible unless there was innate biological fluctuation in the system. No fundamental change in water movement is demanded, and the changes in nutrient chemistry are regarded as indices of the biological changes.

It will be seen that this hypothesis combines elements from hypotheses (2) and (3), but assumes that the ecosystem is

fundamentally unstable and that external influences are less important than supposed previously. Arguments against this hypothesis are largely geographical. There is no doubt that there have been geographical changes in species distribution: for example the east-west shifts in *Sagitta setosa* and the north-south shifts in pilchard. Once we insert a geographical component into this hypothesis there is good reason to suspect a much greater controlling role for external factors, including changes in temperature and water movements.

Conclusions

There is general agreement that the Russell cycle is broadly related to climate change^{2,3,51}. Possibly the lack of satisfactory statistical correlation in detail is due to different response, at different rates of change, by the various biological and chemical components of the ecosystem, some being affected directly, others indirectly. If such is the case no single facet of the climatic change will stand out as the controlling factor, and temperature, water movements, competition and natural fluctuations may all be important. It seems likely that there is an overriding influence of climate in three ways: (i) a hemispheric shift in boundaries of distribution; (ii) direct effects of temperature on the relative competitive advantages of north-south pairs of species in regions of their overlap; and (iii) changes in the local pattern of water circulation in the western half of the Channel, between a predominantly anticyclonic pattern (as in the 1920s and recently) and a predominantly cyclonic pattern (as from 1930 to the 1960s) resulting indirectly from changes in weather and temperature. With this combination of climate related effects there is no need to assume great alteration in net transport through the Channel. The apparent detection of increased transport of Channel species into the southern North Sea⁶⁴ may merely reflect the change in communities in the Western Channel, eventually communicated to the eastern half.

We do not have a long enough series to determine the exact frequency of the Russell cycle, or to judge the suggested fit with the 180-yr solar cycle²⁷. However, historic records show fluctuations in the relative fortunes of the herring and pilchard fisheries off Devon and Cornwall^{16,26}. If these previous fluctuations correspond to changes in the ecosystem similar to those of the Russell cycle, then we may assume that such change is normal for the Western Channel, and that the apparent stability maintained over a period of several years is

in fact a condition of precarious equilibrium. Similar states of equilibrium may exist in other regions, especially near to faunistic boundaries, as off California and Japan⁶⁶. However, we seem to have more detail for the English Channel system, either because it is unduly sensitive, or because it has been studied in a more effective way to reveal the fluctuations.

The degree of sensitivity of the Western Channel ecosystem is underlined by the apparent disparity between the biological fluctuations and the physical and chemical factors that have been monitored. Many species have been replaced by others, and there have been changes in abundance of one or two orders of magnitude, but no obvious trends in salinity can be found, the variation in inorganic phosphate has been less than 25% of the average, and mean annual sea temperature has changed only 0.5°C. Variations in solar luminosity also seem to be small and difficult to relate to changes in climate⁶⁷. A more recent solar index, sunspot umbra/penumbra ratio⁶⁸, also shows only small changes, though these apparently correspond with the secular trend in temperature of the Northern Hemisphere. Yet the economic consequences of the Russell cycle changes have been considerable. In the first phase, cold-water fish of great importance on the UK market (such as herring, cod, haddock, ling, whiting, plaice, dab, lemon-sole) became reduced in number or were replaced by warm-water species (such as pilchard, horse-mackerel and bream) of lesser market appeal⁸. Nowadays, of course, increases in pilchard and horse-mackerel, which are subjected to industrial fishing for meal, might have different economic consequences. Since the beginning of the reversal of the Russell cycle in 1965, some ports in south Devon and south-east Cornwall have seen an increase of nearly an order of magnitude in the number of vessels engaged in fishing. Part of this increase is due to market forces other than fish abundance, but the lesson is still there that almost imperceptible environmental fluctuation may be associated with biological changes of great economic impact. The need for sustained biological monitoring and development of methods of prediction is obvious.

I thank my colleagues at Plymouth, especially G. T. Boalch, E. I. Butler, P. G. Corbin, P. M. Holligan, R. D. Pingree and F. S. Russell for discussions and unpublished data, Linda Maddock for computing and Elizabeth Roberts for help with the literature. I am indebted to Sir Frederick Russell, Dr E. J. Denton and Mr E. I. Butler for critical reading of the manuscript.

- Russell, F. S. *J. mar. biol. Ass. U.K.* **20**, 595-604 (1936).
- Russell, F. S., Southward, A. J., Boalch, G. T. & Butler, E. I. *Nature* **234**, 468-470 (1971).
- Cushing, D. H. & Dickson, R. R. *Adv. mar. Biol.* **14**, 1-122 (1976).
- Raymont, J. E. G. *Plankton and Productivity in the Oceans* (Pergamon, Oxford, 1963).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **19**, 73-82 (1933).
- Corbin, P. G. *J. mar. biol. Ass. U.K.* **28**, 718-722 (1948).
- Holme, N. A. *J. mar. biol. Ass. U.K.* **32**, 1-49 (1953).
- Southward, A. J. *J. mar. biol. Ass. U.K.* **43**, 1-29 (1963).
- Kemp, S. *Rep. Br. Ass. Adv. Sci.* **1938**, 85-101 (1938).
- Atkins, W. R. G. *J. mar. biol. Ass. U.K.* **13**, 700-720 (1925).
- Cooper, L. H. N. *Deep Sea Res.* **31** suppl., 212-223 (1955).
- Cooper, L. H. N. *J. mar. Res.* **14**, 347-362 (1955).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **26**, 605-608 (1947).
- Corbin, P. G. *J. mar. biol. Ass. U.K.* **28**, 271-275 (1949).
- Southward, A. J. *J. mar. biol. Ass. U.K.* **42**, 275-375 (1962).
- Cushing, D. H. *Fish. Invest., Lond. Ser.* **2** **21**, 1-27 (1957).
- Cushing, D. H. *J. mar. biol. Ass. U.K.* **41**, 799-816 (1961).
- Ahlmann, H. W. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer.* **125**, 9-16 (1949).
- Cooper, L. H. N. *J. mar. biol. Ass. U.K.* **37**, 1-3 (1958).
- Southward, A. J. *J. mar. biol. Ass. U.K.* **39**, 449-458 (1960).
- Russell, F. S. *Rep. Trans. Devon. Ass. Advmt Sci.* **85**, 1-17 (1953).
- Southward, A. J. & Crisp, D. J. *J. anim. Ecol.* **23**, 163-177 (1954).
- Blacker, R. W. *Fish. Invest., Lond. Ser.* **2** **20**(10), 1-49 (1957).
- Dickie, L. M. *J. Fish. Res. Bd. Can.* **33**, 313-316 (1976).
- May, R. M. *Nature* **282**, 443-444 (1979).
- Southward, A. J. *J. mar. biol. Ass. U.K.* **54**, 641-649 (1974).
- Southward, A. J., Butler, E. I. & Pennycuik, L. *Nature* **253**, 714-717 (1975).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **18**, 559-574 (1933).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **20**, 147-180 (1935).
- Southward, A. J. *Nature* **249**, 180-181 (1974).
- Llewellyn, J., Macdonald, S. & Green, J. E. *J. mar. biol. Ass. U.K.* **60**, 73-79 (1980).
- Garstang, W. *J. mar. biol. Ass. U.K.* **6**, 274-275 (1901).
- Southward, A. J. & Mattacola, A. D. *J. mar. biol. Ass. U.K.* **60**, 39-44 (1980).
- Cooper, L. H. N. *J. mar. biol. Ass. U.K.* **39**, 173-208 (1960).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **20**, 507-522 (1936).
- Russell, F. S. *J. mar. biol. Ass. U.K.* **53**, 347-355 (1973).
- Fraser, J. H. *J. mar. biol. Ass. U.K.* **41**, 305-312 (1961).
- Wilson, D. P. & Armstrong, F. A. J. *J. mar. biol. Ass. U.K.* **41**, 663-680 (1961).
- Cooper, L. H. N. *J. mar. biol. Ass. U.K.* **23**, 181-195 (1938).
- Harvey, H. W. *The Chemistry and Fertility of Sea Waters* (Cambridge University Press, 1955).
- Butler, E. I., Knox, S. I. & Liddicoat, M. I. *J. mar. biol. Ass. U.K.* **59**, 239-250 (1979).
- Goldman, J. C., McCarthy, J. J. & Peavey, D. G. *Nature* **279**, 210-215 (1979).
- Eppeley, R. W. & Peterson, B. J. *Nature* **282**, 677-680 (1979).
- Boalch, G. T., Harbour, D. S. & Butler, E. I. *J. mar. biol. Ass. U.K.* **58**, 943-953 (1978).
- Armstrong, F. A. J. & Tibbits, S. J. *J. mar. biol. Ass. U.K.* **48**, 143-152 (1968).
- Ford, E. J. *J. mar. biol. Ass. U.K.* **19**, 305-384 (1933).
- Dickie, L. M. *J. Fish. Res. Bd. Canada* **30**, 2496-2506 (1973).
- Garrod, D. J. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer.* **164**, 43-56 (1973).
- Iles, T. D. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer.* **164**, 228-240 (1973).
- Furnestin, M.-L. *Rep. Trav. off. Pêches Marit.* **22**, 211-224 (1958).
- Maddock, L. & Swann, C. L. J. *J. mar. biol. Ass. U.K.* **57**, 317-338 (1977).
- Dietrich, G. *Dtsch. hydrogr. Z.* **3**, 184-201 (1950).
- Colebrook, J. M. *Nature* **263**, 576-577 (1976).
- Colebrook, J. M. *Oceanol. Acta* **1**, 9-23 (1978).
- Colebrook, J. M. & Taylor, A. H. *Deep-Sea Res.* **26A**, 825-850 (1978).
- Kukla, G. J. *et al. Nature* **270**, 573-580 (1977).
- Lincoln, J. V. *NOAA Environm. Data Info. Serv.* **11** (1), 17-19 (1980).
- Wood, K. D. *Nature* **240**, 91-93 (1972).
- Martin, J. H. A. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer.* **162**, 213-219 (1972).
- Worthington, L. V. *Johns Hopkins Oceanogr. Stud.* **6**, 1-110 (1976).
- Lamb, H. H. *Climate, Past, Present and Future* Vol. 1 (Methuen, London, 1972).
- Prandle, D. *J. mar. biol. Ass. U.K.* **58**, 965-973 (1978).
- Carruthers, J. N., Lawford, A. L. & Veley, V. F. C. *Ann. Biol., Copenhagen* **6**, 115-120 (1950).
- Reid, P. C. *Mar. Biol.* **40**, 337-339 (1977).
- Colebrook, J. M., Reid, P. C. & Coombs, S. H. *Mar. Biol.* **45**, 209-213 (1978).
- Birman, I. B. *Vop. Ikhtiol.* **13**, 23-37 (1973).
- Dickie, R. H. *Nature* **280**, 24-27 (1979).
- Hoyt, D. V. *Climatic Change* **2**, 79-92 (1979).
- Southward, A. J. *J. mar. biol. Ass. U.K.* **50**, 689-712 (1970).

ARTICLES

Specific *in vitro* initiation of transcription on conalbumin and ovalbumin genes and comparison with adenovirus-2 early and late genes

B. Wasylyk, C. Kédinger, J. Corden, O. Brison* & P. Chambon†

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS et Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, Strasbourg, France

Specific initiation of transcription of cloned conalbumin and ovalbumin genes has been obtained in a cell-free system in the presence of purified calf thymus RNA polymerase B. Comparison with the adenovirus-2 early (E1A) and major late genes reveals marked variations in transcriptional efficiencies. Specific fragmentation of the conalbumin gene DNA indicates that a short DNA segment 5' to the initiation site is involved in specific transcription.

In prokaryotes, gene expression is controlled, in part, at the level of RNA transcription. The elucidation, at the molecular level, of the regulatory mechanisms which modulate the efficiency with which RNA polymerase recognizes promoter and terminator sequences has been greatly aided by the availability of appropriate mutants and of *in vitro* cell-free systems capable of synthesizing specific RNAs (see refs 1, 2 and refs therein).

In contrast, in eukaryotes, although there is evidence that the expression of at least some genes is regulated at the level of transcription (see refs 3, 4 and refs therein), little is known about the underlying molecular mechanisms. It is firmly established that cells of both higher and lower eukaryotes contain three structurally and functionally distinct classes of DNA-dependent RNA polymerase, which catalyse the synthesis of ribosomal RNA (class A or I), mRNA (class B or II) and tRNA and 5S RNA (class C or III) (reviewed in refs 5, 6). However, until recently, all attempts to demonstrate convincingly specific *in vitro* transcription of purified DNAs from viral or cellular origin with the three classes of purified RNA polymerases have failed (reviewed in refs 3, 5, 6), suggesting that additional chromatin or nuclear components are necessary.

An important advance in the identification of such components was made by Wu⁷, who established a cell-free system in which the virus-associated (VA) RNA genes of purified adenovirus-2 DNA is selectively transcribed by RNA polymerase C. This has led to the demonstration of the existence of factors required for specific transcription by RNA polymerase C (ref. 8 and refs therein). More recently, using a similar system, Weil *et al.*⁹ have demonstrated accurate initiation of transcription by purified RNA polymerase class B at the major late adenovirus-2 promoter. On the other hand, it has only recently become possible to make a useful search for chromatin or other cellular components necessary for purified RNA polymerase B to transcribe accurately genes encoding cellular mRNA (discussed in refs 3, 5). The main reasons for this were the complexity of eukaryotic genomes and the lack of information regarding specific transcription units. The situation has radically changed with the advent of molecular cloning and fast DNA sequencing methods. The structure of

several messenger-coding genes has been established and putative promoter regions have been positioned and sequenced (refs 10–13 and refs therein).

We report here that, using the cell-free system of Wu⁷ and cloned DNA templates^{11,12}, purified RNA polymerase class B can accurately initiate transcription *in vitro* at the 5' ends of the transcription units of the conalbumin and ovalbumin genes [whose expressions are hormonally controlled in the chicken (see refs 11, 12 and refs therein)]. However, a comparative study of these two genes, and of early E1A and major late adenovirus-2 genes, indicates that different genes are transcribed with very different efficiencies in this system. Our experiments also demonstrate that at least part of the conalbumin DNA sequences required for specific initiation of transcription are situated in the region where presumptive promoter sequences have been previously positioned¹².

Specific initiation of transcription on the conalbumin gene

We have previously reported that the DNA sequences coding for conalbumin mRNA (con-mRNA) are contained in three *EcoRI* fragments, 'b', 'c' and 'a' (in the order of transcription)¹². Sequence studies have shown that the cap site of con-mRNA is coded by the 5' end of exon 1, which is contained in fragment *EcoRI* 'b' (Fig. 1B; see also Figs 1, 5 in ref. 12). Two observations suggest that the cap site could correspond to the site of initiation of transcription. First, the largest con-mRNA precursor molecule is the same size as the DNA segment containing the con-mRNA coding sequences (ref. 12, and M. LeMeur, A. Krust and S. McKnight, personal communications). Second, about 25 base pairs upstream from the cap site, there is an A+T-rich sequence which might correspond to a promoter site (refs 10–13 and refs therein). Particularly striking is the similarity between this conalbumin sequence and the corresponding sequence of the major late adenovirus-2 gene (see Fig. 7B in ref. 12). These observations prompted us to study whether the cell-free system⁷ which was used by Weil *et al.*⁹ to demonstrate specific *in vitro* initiation of transcription at the *in vivo* cap site of the major late adenovirus-2 genes could also be used to transcribe selectively cellular genes with purified RNA polymerase B.

To detect specific initiation of transcription on the conalbumin gene, we used the truncated template assay⁹, which contains the S100 transcription cell-free system of Wu^{8,14} prepared from HeLa cells, purified calf thymus RNA

*Present address: Stanford University, Department of Biochemistry, Stanford, California 94305.

†To whom correspondence should be addressed.

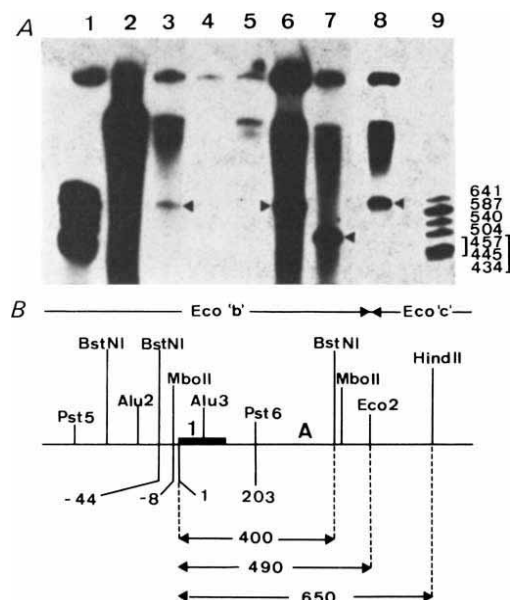


Fig. 1 Electrophoretic analysis of RNA synthesized on conalbumin 'truncated' templates. **A**, RNA was synthesized for 60 min at 30°C in a standard reaction mixture (50 µl) containing (final concentrations) 25 µM [α - 32 P]GTP (9,000 c.p.m. pmol $^{-1}$), 600 µM ATP, 600 µM CTP, 600 µM UTP, 10 mM Tris-HCl pH 7.9, 7.5 mM MgCl $_2$, 50 mM KCl, 0.25 mM dithiothreitol, 0.150 units of calf thymus RNA polymerase B (glycerol gradient fraction) 15 , 25 µl of HeLa cell S100 extract $^{7-9}$ and conalbumin DNA (see below). After synthesis, the reaction mixture was diluted with 450 µl of a solution containing 50 mM sodium acetate pH 5.0, 0.5% SDS and 50 µg ml $^{-1}$ yeast tRNA (final concentrations), extracted once with phenol, twice with chloroform, made 0.15 M in ammonium acetate and precipitated with two volumes of ethanol. The RNA pellet was dissolved in 30 µl formamide buffer, denatured for 5 min at 65°C, chilled on ice and analysed on a 5% acrylamide-urea gel 41 . The incubations corresponding to lanes 2-6 and 8 contained 6 µg ml $^{-1}$ of a 5,700-base pair *HindII* fragment prepared from cloned conalbumin DNA 12 and containing 2,700 base pairs of conalbumin DNA [from the *HindII* site in intron A (see below) to the *KpnI* site of the *Eco* 'b' fragment] linked to 3,000 base pairs of pBR322 [from the *PstI* site to the *HindII* site at position 652 (ref. 42)]. The incubations were modified as follows: lane 2: no added S100 extract; lane 3: no calf thymus polymerase B; lane 4: no DNA; lane 5: with 1 µg ml $^{-1}$ α -amanitin; lane 6: standard reaction; lane 7, 4.2 µg ml $^{-1}$ of purified conalbumin *Eco* 'b' fragment 12 instead of *HindII* fragment. Lanes 1 and 9 contain the same amount of 32 P-labelled DNA size markers. Lanes 8 and 9 are the same as lanes 6 and 1, respectively, but less exposed. Arrowheads point to bands discussed in the text. **B**, Restriction enzyme map around exon 1 of the conalbumin gene. Sites *Pst5*, *Alu2*, *Alu3*, *Pst6* and *Eco2* have been described previously 12 . The position of the *BstNI*, *MboII* and *HindII* sites was either deduced from the DNA sequence 12 or established by restriction enzyme mapping (unpublished results). *Eco* 'b' and *Eco* 'c' refer to *EcoRI* conalbumin fragments 'b' and 'c' (ref. 12). The heavy line 1 and A, correspond to exon 1 and intron A, respectively 12 . The figures below the restriction map refer to the number of nucleotides from the cap site (nucleotide 1) and correspond to the restriction enzyme cutting sites on the non-coding strand (negative numbers are upstream from the cap site with respect to the direction of transcription) 12 . The distances (in base pairs) indicated by arrows are from size analysis of restriction fragments.

polymerase B (ref. 15) and either the isolated conalbumin *Eco* 'b' fragment or an '*HindII*-cut' conalbumin plasmid as templates (see Fig. 1B and Fig. 1A legend). By electrophoretic analysis (Fig. 1A), discrete bands of 650 (lanes 6 and 8) and 500 (lane 7) nucleotides were found with the '*HindII*' and *Eco* 'b' templates, respectively. These correspond to the sizes expected if transcription starts at the 5' end of exon 1 (Fig. 1B). Similarly, transcription of the isolated *Pst5*-*Pst6* fragment (Fig. 1B) gave mainly a 200-nucleotide long RNA (not shown), confirming that transcription is occurring predominantly from the coding strand of the conalbumin gene. The comparison of lanes 6 and 7 indicates that most of the nonspecific transcription seen in lane 6 comes from the pBR322 sequences present in the '*HindII*-truncated' template. As shown in lane 2, the S100 extract is necessary for specific transcription (no

discrete bands were observed with exposure for a shorter time), although, as previously observed in the adenovirus case 9 , its addition depressed total RNA synthesis (compare Fig. 1, lanes 2 and 6, and unpublished observations). The synthesis of specific RNA is dependent on the addition of the conalbumin template (lane 4) and is catalysed by RNA polymerase B [as shown by inhibition at low concentrations of α -amanitin (lane 5)]. A small fraction of the specific synthesis is due to the RNA polymerase B which is present in the S100 extract (lane 3, also see below).

Transcription of the isolated *EcoRI* fragment 'b' (Fig. 1A, lane 7) suggests that a large fraction of the RNA synthesized on conalbumin DNA is, in fact, initiated at the 5' end of exon 1. This is demonstrated in Fig. 2, where the RNA synthesized on *EcoRI* fragment 'b' hybridized much more strongly to the *MboII*-*Pst6* (positions -8 to +203) (lane 2) than to the *Pst6*-*MboII* (lane 4) fragment (see Fig. 1B). In contrast, RNA randomly synthesized on denatured *EcoRI* fragment 'b' in the absence of S100 extract hybridized equally well to both fragments (lanes 1, 3).

The above results suggest very strongly that transcription of the conalbumin DNA is initiated on the coding strand near the 5' end of exon 1. The modified *S* $_1$ mapping technique of Weaver and Weissmann 16 was used to position the 5' end of the *in vitro* synthesized RNA. Unlabelled RNA synthesized on the *HindII*-truncated template (Fig. 3A, lanes 2-4) or hen oviduct poly(A) $^+$ RNA (lanes 5-7) were hybridized to the *Alu2*-*Alu3* fragment (Fig. 1B) which had been labelled with 32 P at the 5' ends and, after *S* $_1$ nuclease digestion, the resistant labelled DNA was run on a sequencing gel in parallel with a G+A sequence ladder of the *Alu3*-*MboII* fragment (+62 to -8, Fig. 1B). Both *in vivo* and *in vitro* RNAs gave the same digestion patterns, of which the lowest bands (arrowheads) in each case migrate with the same mobility as the G at position -1 in the sequencing ladder. Therefore, the 5' ends of the two

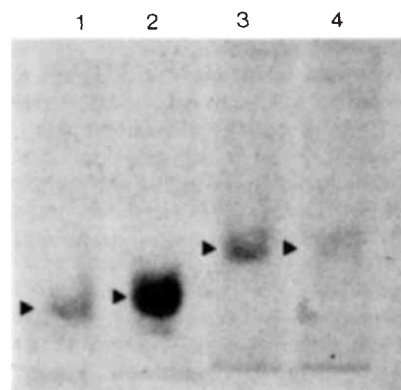


Fig. 2 Hybridization of RNA synthesized *in vitro* on conalbumin *Eco* 'b' fragment to conalbumin DNA fragments surrounding the cap site. The two *MboII* fragments from cloned *Pst5*-*Pst6* conalbumin DNA (Fig. 1B) were separated on a 5% acrylamide gel, eluted, re-run on separate slots of a 3% agarose gel and transferred to diazobenzoyloxymethyl (DBM) paper 43 . RNA which had been synthesized either on the native conalbumin *Eco* 'b' fragment (in a twofold standard reaction) as described in Fig. 1A legend, or on the heat-denatured fragment in the absence of S100 extract (standard reaction), was treated with DNase I, extracted and precipitated as described in Fig. 3A legend. The RNA pellet was redissolved in 75 µl of formamide dextran buffer, rapidly cooled and hybridized to the DNA bound to the DBM paper for 12 h at 42°C, as described by Wahl *et al.* 43 . The paper was washed with two changes of 2 $\times$ SSC for 30 min each, treated with 20 µg of pancreatic RNase (Sigma) and 10 units of T $_1$ (Sigma) in 1 ml of 2 $\times$ SSC for 1 h at 20°C, and washed twice for 20 min at 20°C with 2 $\times$ SSC containing 0.1% SDS and then twice for 45 min at 50°C with 0.1 $\times$ SSC containing 0.1% SDS. Lane 1: DBM-paper-bound *MboII*-*Pst6* fragment (Fig. 1B) and RNA synthesized on denatured DNA; lane 2: DBM-paper-bound *MboII*-*Pst6* fragment and RNA synthesized on native *Eco* 'b' fragment; lane 3: as lane 1, but with DBM-paper-bound *Pst5*-*MboII* fragment; lane 4: as lane 2, but with DBM-paper-bound *Pst5*-*MboII* fragment. Arrowheads indicate bands discussed in the text.

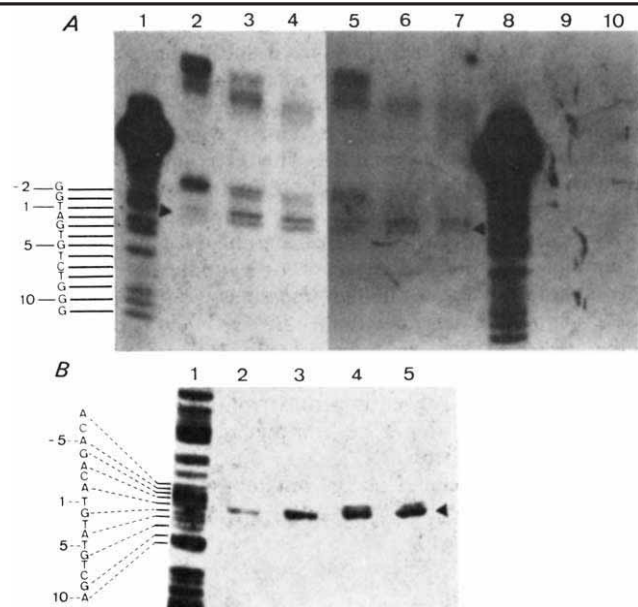


Fig. 3 Localization of the 5' ends of RNA synthesized *in vitro* on conalbumin and ovalbumin genes by S_1 nuclease mapping. **A**, Conalbumin RNA. Lanes 1 and 8: G + A sequence ladders⁴¹ prepared from the *Mbo*II (position -9) to *Alu*I (position 62) fragment (Fig. 1B) labelled at the *Alu*I site. The sequence¹² around the conalbumin cap site (position 1) is shown in the margin. Lanes 2-4: RNA was synthesized *in vitro* (2.5 times the standard reaction but with unlabelled nucleotides) on the conalbumin *Hind*II fragment (see Fig. 1A), purified as described in Fig. 1A, dissolved in 125 μ l 50 mM Tris-HCl, pH 7.9, and 10 mM $MgCl_2$ and digested with 50 μ g ml⁻¹ RNase-free DNase I (ref. 44) for 10 min at 37 °C. After extraction with phenol chloroform and chloroform, the RNA was ethanol precipitated with 0.1 μ g of the *Alu*2-*Alu*3 fragment (Fig. 1B) labelled with ³²P (specific activity ~50,000 c.p.m. pmol⁻¹ of 5' ends), redissolved in 37.5 μ l of 80% formamide, 40 mM PIPES pH 6.5, 0.4 M NaCl and 1 mM Na-EDTA, heated to 85 °C for 5 min and incubated for 12 h at 43 °C. After cooling at -30 °C the mixture was diluted 10-fold, adjusted to 30 mM sodium acetate (pH 4.5), 3 mM $ZnSO_4$, 0.4 M NaCl and 6.7 μ g ml⁻¹ sonicated calf thymus DNA and incubated with 4,000 units of S_1 nuclease (Miles) at 25 °C. After 1 h (lane 2), 4 h (lane 3) and 7 h (lane 4) Tris-HCl, pH 7.9, and Na-EDTA, pH 7.0, were added to 300 mM and 15 mM (final concentrations), and after phenol-chloroform extraction and ethanol precipitation the samples were analysed on a 12% acrylamide-urea sequencing gel⁴¹. Lanes 5-7: the conditions were as in lanes 2-4, except that 1.2 μ g of hen oviduct poly(A)⁺ RNA was hybridized with the *Alu*2-*Alu*3 probe; lanes 5-7: 1, 4 and 7 h of S_1 nuclease digestion, respectively. Lanes 9, 10: the conditions were as in lane 3 except that adenovirus major late gene DNA (see Fig. 4 legend) replaced conalbumin DNA (lane 9) or 1 μ g ml⁻¹ α -amanitin was included in the incubation (lane 10). Lanes 1-4 and 5-10 are from the same sequencing gel, but were exposed for different times. **B** Ovalbumin RNA. Lane 1, G + A sequence ladder prepared from the *Pvu*II-*Hind*III (*Hind*3 site, see ref. 11) ovalbumin DNA fragment. The sequence¹¹ around the ovalbumin cap site (position 1) is shown in the margin. Lanes 2, 3: the conditions were as in A, lanes 2-4, with the following two exceptions: *Pst*4-*Eco*6 (ref. 11) ovalbumin DNA plasmid (*Pst*4-*Eco*6 inserted between the *Pst*I and *Eco*RI sites of pBR322) was linearized with *Eco*RI, and the probe was the *Pst*4-*Eco*6 ovalbumin plasmid (5 μ g per hybridization) cut with *Hind*III and labelled with ³²P (specific activity ~500,000 c.p.m. pmol⁻¹ of 5' ends) (lanes 2 and 3: 6.5 h and 4 h of S_1 nuclease digestion, respectively). Lanes 4, 5: the conditions were as in A, lanes 2-4, except that 100 μ g of hen oviduct poly(A)⁺ RNA and 1 μ g of labelled probe were hybridized in 25 μ l of formamide buffer. S_1 nuclease digestion was for 4 h (lane 5) and 6.5 h (lane 4) with 1,600 units of S_1 . These quantities of poly(A)⁺ RNA and probe gave a similar amount of total S_1 nuclease-resistant counts as their *in vitro* counterpart (B, lanes 2, 3). Arrowheads indicate bands discussed in the text.

RNAs are identical and correspond to the T, numbered 1, which was previously identified as coding for the first nucleotide of con-mRNA¹². (Two control experiments in lanes 9 and 10, described in Fig. 1 legend, show that no bands are present when the incubation was carried out in the absence of conalbumin DNA or in the presence of a low concentration of α -amanitin.) The presence of the lowest doublet bands in lanes 3, 4, 6 and 7 may be due to incomplete S_1 nuclease digestion related to capping of the RNAs (see below), whereas the upper

bands in lanes 2, 3, 5 and 6 are probably due to the secondary structure(s) of the DNA probe (see the DNA sequence in ref. 12).

All these results are compatible with specific *in vitro* initiation of transcription at the 5' end of the conalbumin gene. This conclusion is further supported by analysis of RNase T₁ digests of the *in vitro* synthesized RNA, which shows the presence of the expected terminal T₁ oligonucleotide, both in the capped and uncapped forms (C.K. *et al.*, in preparation). Weil *et al.*⁹ have also observed capping of the RNA synthesized *in vitro* from the major late adenovirus-2 gene.

Specific, but less efficient, transcription of ovalbumin and early E1A adenovirus-2 genes

As shown in Fig. 4, specific transcription was also observed with two 'truncated' templates containing the 5' region of the ovalbumin gene and cut with either *Eco*RI or *Hind*III at the *Eco*6 or *Hind*3 sites, respectively (see Fig. 1c in ref. 11). The two distinct bands [400 nucleotides (lane 4) and 220 nucleotides (lane 5)] are expected if initiation of transcription is taking place at the 5' end of the ovalbumin leader coding sequence¹¹. The discrete bands which are seen above the 220-nucleotide RNA in lane 5 do not correspond to specific run-off transcripts (that is, transcription from specific sites to the end of the truncated template), as bands with identical size were observed on longer exposure of lane 4. In an experiment similar to that described above for the conalbumin transcript, S_1 nuclease mapping was used to localize more precisely the 5' end of the *in vitro* synthesized RNA (Fig. 3B). *In vivo* hen oviduct poly(A)⁺ RNA or *in vitro* RNA synthesized on the truncated *Eco*6 template were hybridized to a probe 5'-labelled at the *Hind*3 site. After nuclease S_1 digestion, the resistant labelled DNA fragments were run on a sequencing gel together with a G + A sequence ladder around the 5' end of the ovalbumin gene. The same bands were observed with *in vivo* (lanes 4, 5) and *in vitro* (lanes 2, 3) RNAs, but not in control experiments run in the absence of ovalbumin DNA or in the presence of α -amanitin (not shown). The position of the resistant bands corresponds to the A at position -1 of the ovalbumin coding strand, suggesting that the *in vitro* synthesized RNA was initiated at the T (at position 1 in the ovalbumin gene) which codes for the first nucleotide of ovalbumin mRNA¹¹. Our results confirm those of LeMeur (personal communication) who, with the same *Hind*3 5'-labelled probe, showed that the 5' ends of the ovalbumin mRNA precursor molecules map at the cap site, and found no evidence for RNA molecules initiated *in vivo* upstream from that site. It seems, therefore, that the ovalbumin gene is also specifically transcribed in the *in vitro* cell-free system. However, it is clear, from a comparison of lanes 2 and 4 in Fig. 4, that the ovalbumin gene is transcribed with a much lower efficiency than the conalbumin gene, although, for both genes, care was taken to carry out the reactions at optimum DNA concentrations. The efficiency of transcription of the ovalbumin gene was not increased by using the purified *Pst*4-*Eco*6 fragment¹¹ as a template (not shown).

The difference in transcriptional efficiency of conalbumin and ovalbumin genes prompted us to compare the transcription of these genes with two adenovirus genes, the major late, previously studied by Weil *et al.*⁹, and the early E1A (ref. 17), whose 5'-end regions have been cloned in our laboratory (see Fig. 4 legend). The major late adenovirus gene template, truncated at the 18.2 *Sma*I site¹⁸, gave a run-off transcript of about 560 nucleotides (lane 6), as expected from the results of Weil *et al.*⁹. Comparison with the 650-nucleotide run-off transcript of the truncated *Hind*II conalbumin template (lane 7) showed that, at optimum DNA concentrations, the major late adenovirus gene was a somewhat better template

than the conalbumin gene (variations were observed from one experiment to another, but in general the intensity of the late adenovirus band was approximately twice that of the conalbumin). Specific transcription of the adenovirus-2 early E1A gene was also observed in the cell-free system. Two truncated templates were used (see Fig. 4 legend), one cut at the *Sma*I site, which is at position 1,009 from the left end of the adenovirus genome (2.9 map units)¹⁷, the other at an *Ava*I site, which we mapped in our adenovirus-2 DNA at position 758 (numerology of ref. 19). Because the 5' end of the E1A mRNAs maps at position 499 (see ref. 17), two run-off transcripts of 510 and 260 nucleotides, respectively, were expected, assuming that transcription starts at the cap site of E1A mRNAs. The two predicted run-off transcripts are, in fact, observed in lanes 8 and 9 of Fig. 4.

It is clear that the *in vitro* transcription of the early adenovirus E1A templates is much less efficient than that of the conalbumin and major late adenovirus genes, and in this respect is similar to that of the ovalbumin gene. Interestingly, the X gene, which evolved from the same ancestral gene as the ovalbumin gene (ref. 20, and R. Heilig and J. L. Mandel, personal communication), is transcribed *in vitro* with about the same efficiency as the ovalbumin gene (not shown). The reason that the shorter run-off transcripts of the ovalbumin (lane 5) and early EA1 adenovirus gene (lane 9) are less intense than expected from their reduced size is unknown (note in Fig. 4 legend that lanes 5 and 9 were exposed for a longer time), but similar observations were made for the short run-off transcripts of conalbumin and major late adenovirus genes.

The observed marked variation in the transcriptional efficiency of different genes raised the possibility that RNA polymerase B or the factor(s) contained in the S100 extract, or both, could have different affinities for various genes. This possibility was tested with the conalbumin and ovalbumin

genes. In the experiment shown in lane 3 of Fig. 4, the two incubation mixtures used for lanes 2 and 4 were mixed. Both the conalbumin and the ovalbumin run-off transcripts are observed in this mixed incubation (arrowheads in lane 3; although the ovalbumin band is faint it was consistently seen on the original autoradiograms). This experiment was carried out at optimum DNA concentration for both conalbumin and ovalbumin specific transcription. The same result was obtained in the presence of four times this amount of conalbumin and ovalbumin templates. These observations indicate that there is no competition between the two templates.

Several possibilities could be invoked to explain these results. First, different factors, specific for different genes, could be present in the S100 extract. Second, the same factor(s) could be used equally well for transcription of the two genes, but the rate limiting step is beyond that requiring the factor(s). Third, the factor(s) present in the S100 extract is not required by the ovalbumin gene. This last possibility is excluded, as the S100 extract was required for specific transcription of all of these genes (not shown).

Some sequences, situated 5' to the cap site, are required for specific *in vitro* transcription

The existence of related sequences (see above and refs 10–13) 5' to the *in vitro* initiation site on the conalbumin, ovalbumin and adenovirus-2 genes, led us to investigate whether these sequences are required for specific *in vitro* transcription. In this respect, the conalbumin gene is especially convenient because there is a *Bst*NI site at position -44 and an *Mbo*II site at position -8 (Fig. 1B). As shown in Fig. 5A, a *Bst*NI-digested conalbumin *Eco* 'b' fragment gave the expected run-off transcript of 400 nucleotides [lane 2; the efficiency of specific transcription was the same as on intact *Eco* 'b' fragment (not shown)], whereas this transcript was absent with a conalbumin *Eco* 'b' template cut with both *Bst*NI and *Mbo*II (lane 3). (No attempt has been made to identify the nature of the minor bands present in lanes 2 and 3 because 'non-specific' bands are seen in all these *in vitro* transcription assays.) Similarly, the isolated *Pst*5-*Pst*6 fragment (Fig. 1B), digested with *Bst*NI, gave a run-off band of about 200 nucleotides, whereas no distinct band was observed when the *Pst*5-*Pst*6 fragment was cut with *Mbo*II (not shown). *S*₁ mapping experiments, similar to those reported in Fig. 3A, showed that the 5' end of the specific transcript obtained on the 'BstNI-cut' template, maps at the position coding for the first nucleotide of con-mRNA (B.W., unpublished result).

To exclude the possibility that the above results could be influenced by the absence of DNA upstream from position -44 when specific synthesis is retained, and upstream from position -8 when it is lost, we have cloned the *Bst*NI (-44) to *Pst*I (+203) and the *Mbo*II (-8) to *Pst*I (+203) fragments in the *Bam*HI site of pBR322 (see Fig. 5 legend and Fig. 5C). The recombinant clones in the orientation shown in Fig. 5C (a, b) were cut with either *Bst*NI or *Hind*III and transcribed *in vitro*. As shown in Fig. 5B, the expected run-off transcripts are present (lanes 2, 3) when the recombinant DNA contains the conalbumin sequence up to position -44, but are absent (lanes 4, 5) when the conalbumin sequences are missing upstream from position -8. Similar results were obtained with templates where the conalbumin fragments were inserted in the opposite orientation (B.W., unpublished results).

From these results we conclude that the sequences which are situated upstream from position -44 are not required for specific *in vitro* transcription of the conalbumin gene in this cell-free system. In agreement with this conclusion, we have observed (J.C., unpublished result) that the sequences located upstream from position -48 with respect to the cap site are not required for specific *in vitro* transcription of the major late adenovirus-2 gene. On the other hand, our results demonstrate

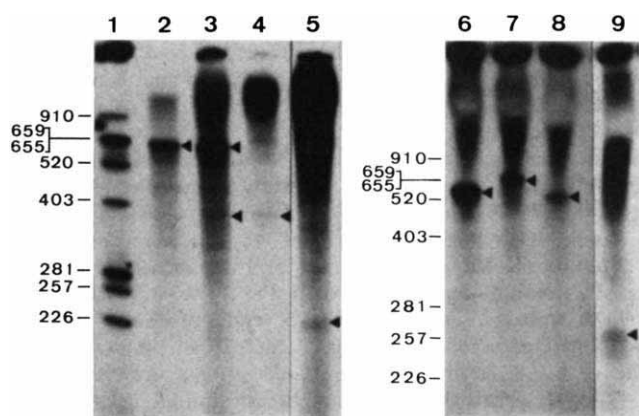


Fig. 4 Comparison of *in vitro* specific RNA transcripts synthesized on conalbumin, ovalbumin and adenovirus-2 DNA templates. RNA was synthesized and extracted as described in Fig. 1A legend, but using different DNA templates. DNA concentrations were chosen to give maximal incorporation into the specific RNA transcripts (see text). Lane 2: $12 \mu\text{g ml}^{-1}$ of *Hind*III conalbumin DNA fragment (5.7 kilobases) (see Fig. 1A legend); lane 4: $11 \mu\text{g ml}^{-1}$ of ovalbumin *Pst*4-*Eco*6 plasmid (5.3 kilobases) linearized with *Eco*RI (see Fig. 3B legend); lane 3: reaction mixtures, equivalent to lanes 2 and 4, respectively, mixed before incubation; lane 5: $11 \mu\text{g ml}^{-1}$ of ovalbumin *Pst*4-*Eco*6 plasmid linearized with *Hind*III (see Fig. 3B legend); lane 6: $20 \mu\text{g ml}^{-1}$ of a *Sma*I [map position 18.2 (ref. 18)] cleaved recombinant plasmid (6.7 kilobases) consisting of the *Bal*I E fragment of adenovirus-2 DNA (map position 14.7–21.5 on the adenovirus-2 genome) integrated into the *Eco*RI site of pBR322; lane 7: $16 \mu\text{g ml}^{-1}$ of the conalbumin *Hind*III fragment; lane 8: $24 \mu\text{g ml}^{-1}$ of a *Sma*I-cleaved recombinant plasmid (5.7 kilobases) consisting of the left-most *Xba*I fragment of adenovirus-2 DNA (map position 0–3.85, see ref. 17) integrated into the *Eco*RI site of pBR322 (to be published elsewhere); lane 9: $36 \mu\text{g ml}^{-1}$ of the same recombinant DNA as in lane 8, but cleaved with *Ava*I (see text); lane 10: ^{32}P -labelled DNA fragment size markers. Lanes 1–5, and 6–9 correspond to two different gels. Lanes 5 and 9 were exposed for a longer time. Arrowheads indicate the bands described in the text.

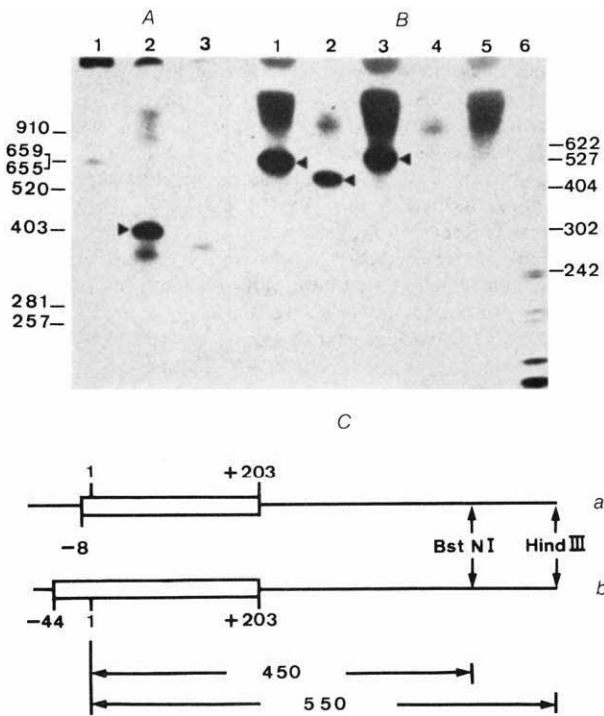


Fig. 5 Effect of deletion in the 5'-end region of the conalbumin gene on its specific transcription. **A**, The excised conalbumin *Eco* 'b' fragment was digested with *Bst*NI (lane 2) or *Bst*NI and *Mbo*II (lane 3) (see Fig. 1), purified by two phenol extractions, two ether extractions, ethanol precipitation and dialysis against 50 mM Tris-HCl, pH 7.9, and 1 mM Na-EDTA. Transcription was done at $16 \mu\text{g ml}^{-1}$ template as described in Fig. 1A legend. Lane 1: ^{32}P -labelled DNA fragment size markers. **B**, The conalbumin DNA fragments from *Bst*NI (-44) to *Pst*I (+203) (lanes 2 and 3) and *Mbo*II (-8) to *Pst*I (+203) (lanes 4, 5) (see Fig. 1 and part C) were repaired with DNA polymerase I and cloned in pBR322 which had been linearized with *Bam*HI and repaired with DNA polymerase. DNA from clones containing the conalbumin gene fragment (in the orientation shown in C) was digested with either *Hind*III (lanes 3, 5) or *Bst*NI (lanes 2, 4), purified as described in A and transcribed *in vitro* at $10 \mu\text{g ml}^{-1}$ template (Fig. 1A). Lane 1: transcription with $8 \mu\text{g ml}^{-1}$ purified *Eco* 'b' fragment (see Fig. 1). Lane 6: ^{32}P -labelled DNA fragment size markers. **C**, Restriction enzyme maps of the cloned *Mbo*II (-8) to *Pst*I (+203) (a) and *Bst*NI (-44) to *Pst*I (+203) (b) conalbumin gene fragments. The figures refer to the number of nucleotides from the cap site (position 1) and correspond to restriction enzyme cutting sites on the non-coding strand (negative numbers are upstream from the cap site with respect to the direction of transcription). The open lines correspond to conalbumin gene DNA and the single lines to pBR322 DNA. The lengths (in base pairs) indicated by arrows correspond to distances from the cap site to the corresponding restriction enzyme cutting site. Arrowheads in A and B correspond to bands discussed in the text.

that there is no specific transcription when the DNA located upstream from position -8 of the conalbumin gene is deleted.

Influence of the origin of RNA polymerase on specific transcription

The sequence similarities which have been observed, in both higher and lower eukaryotes, upstream from the region coding for the 5' end of mRNAs (refs 10-13 and refs therein, refs 21, 22) and the structural similarities between the eukaryotic RNA polymerases belonging to class B (refs 5, 6), raise the question of whether specific *in vitro* transcription can be achieved irrespective of the origin of the polymerase B. Figure 6 shows that the conalbumin gene was specifically transcribed with the same efficiency by both purified calf thymus¹⁵ (lane 2) and hen oviduct²³ (lane 3) RNA polymerase B. In contrast, purified *Drosophila*²⁴ (lane 4), yeast²⁵ (lane 5) and wheat-germ²⁶ (lane 7) RNA polymerases B did not significantly stimulate the specific transcription above the level obtained with the endogenous polymerase B present in the S100 extract (lane 1).

This was supported by the complete disappearance of specific transcription when α -amanitin was added to the yeast incubation (lane 6) at a concentration which does not inhibit yeast RNA polymerase B (ref. 27). No specific transcription above the endogenous level was observed with *Escherichia coli* RNA polymerase (lanes 8, 9). Similar results were obtained when the ovalbumin and the late adenovirus-2 genes were transcribed with the various RNA polymerases B (the lack of specific transcription of the major late adenovirus-2 gene by the wheat-germ RNA polymerase B has been reported previously⁹). In no case did we observe specific transcription using either purified calf thymus RNA polymerase A (ref. 28) or the endogenous RNA polymerase C present in the S100 extract (see Fig. 6, lanes 6, 9, and our unpublished results).

Note that all the enzymes used in the incubations shown in Fig. 6 gave comparable amounts of RNA synthesis when incubated in identical conditions on commercial calf thymus DNA in the absence of S100 extract, and that the specific synthesis which is obtained on the most efficiently transcribed templates (major late adenovirus-2 or conalbumin genes) represents at most 1% of the synthesis which is achieved on calf thymus DNA. This low efficiency of specific transcription by the added purified RNA polymerase B raises the question of whether the endogenous enzyme B, which did not go through all the purification steps, is more efficient in promoting specific transcription. This is apparently not the case, as titration of the S100 endogenous RNA polymerase B with ^3H -amanitin²⁹ indicated that the S100 extract added to each incubation contains about one-seventh the amount of added purified polymerase B (not shown). Clearly, comparison of lanes 1 and 2 of Fig. 6 indicates that the endogenous polymerase B is not more efficient for specific transcription.

Conclusions

Our present results suggest very strongly that specific transcription of cellular genes, transcribed *in vivo* by RNA polymerase B, can be achieved *in vitro* with cloned DNA templates and purified polymerase in the presence of the S100 extract of Wu and Zubay¹⁴. Initiation of *in vitro* transcription seems to be accurate, taking place at the same site as *in vivo*, as there is no evidence that initiation occurs *in vivo*, on the conalbumin or the ovalbumin genes, from a site located upstream from the cap site (see above). Although we cannot

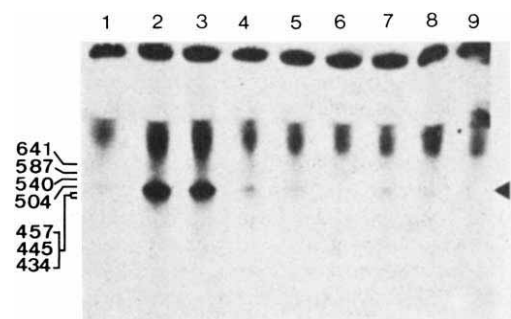


Fig. 6 Effect of different exogenous RNA polymerases on specific *in vitro* transcription of the conalbumin gene. RNA was synthesized on the conalbumin *Eco* 'b' fragment ($12 \mu\text{g ml}^{-1}$) and extracted as described in Fig. 1A legend, but with various RNA polymerases. Lane 1: no added RNA polymerase; lane 2: 0.102 units of purified calf thymus RNA polymerase B (see Fig. 1A legend); lane 3: 0.129 units of purified oviduct RNA polymerase B (fraction HA, ref. 23); lane 4: 0.110 units of purified *Drosophila* RNA polymerase B (ref. 24, and A. Krämer, personal communication); lane 5: 0.100 units of purified yeast RNA polymerase B (ref. 25); lane 6: as in lane 5, but in the presence of $0.1 \mu\text{g ml}^{-1}$ α -amanitin; lane 7: 0.130 units of purified wheat-germ RNA polymerase B (ref. 26); lane 8: 0.140 units of purified *E. coli* holoenzyme²⁵; lane 9: same as lane 8, but in the presence of $1 \mu\text{g ml}^{-1}$ α -amanitin. One unit of RNA polymerase corresponds to the activity measured in the conditions described previously¹⁵ in the presence of 3 mM Mn^{2+} and 100 mM ammonium sulphate. The arrowhead indicates the bands described in the text.

unequivocally rule out the possibility that the specific RNAs seen *in vitro* could result from processing of transcripts initiated upstream from the cap site, this seems very unlikely, at least in the case of conalbumin, where the sequences upstream from position -44 can be deleted without impairing the specific transcription, and specific 5'-terminal uncapped and capped T₁ oligonucleotides have been found.

The observation that the DNA located in the conalbumin gene between positions -8 and -44 is required for specific transcription is in agreement with the proposal that the A+T-rich 'Goldberg-Hogness box' (refs 21 and 10-13, 22, 30 and refs therein) is part of at least one class of promoter site for RNA polymerase B. In this respect, it is striking that the major late adenovirus-2 and the conalbumin genes, which have the same sequence from positions -21 to -32 (ref. 12), are transcribed *in vitro* with about the same efficiency, whereas the ovalbumin, early E1A adenovirus and X genes, which have A+T-rich regions of different sequences, were transcribed with lower efficiencies. Our results demonstrate that the sequences which are situated upstream from position -44 of the conalbumin gene are not required for specific *in vitro* initiation. Obviously, this does not exclude the possibility that some of these sequences could be required *in vivo* and involved in initiation and/or regulation of transcription by interacting with other initiation or regulatory factors. In this respect, it is interesting that homologies have been found by comparing the sequences of several genes upstream from position -44 (ref. 13).

There seem to be differences in the location (with respect to the 5' end of the transcript) of at least some of the sequences necessary for specific initiation by RNA polymerases B and C. It has been shown that up to 50 base pairs into a 5S RNA gene can be replaced without substantially affecting initiation of transcription by RNA polymerase C (see refs 31-33, but refs 34, 35 for different results using the VA RNAI adenovirus gene or the *Xenopus* tRNA₁ Met gene as templates). Obviously, our present results do not exclude the possibility that sequences situated downstream from position -8 are also required for specific transcription by RNA polymerase B. In fact, we have found that the *Mbo*II-*Pst*5 conalbumin fragment (see Fig. 1B), when inserted in the *Bam*HI site of pBR322, does direct specific initiation of transcription (results not shown).

Almost nothing is known of the nature and mechanism of action of the factor(s) present in the S100 extract and responsible for the specific transcription (see also ref. 9). However, in view of the overall inefficiency of the system, other factors (either catalytic or structural) are probably required to account for the *in vivo* rates of transcription. Addition to the incubation mixtures of the RNA polymerase B stimulatory factor isolated by Stein and Hausen³⁶ did not increase the rate of specific transcription (H. Stein and C. Kédinger, unpublished results). It is unlikely that the overall inefficiency is related to the use of heterologous systems, as RNA polymerase B purified from hen oviduct was not more effective than the purified calf thymus polymerase B in transcribing the chicken conalbumin or ovalbumin genes (Fig. 6). In addition, transcription of the major late adenovirus-2 gene, in homologous or heterologous systems (ref. 9, and our present results), does not seem to be substantially more efficient than that of the conalbumin gene in a heterologous system. The fact that the *Drosophila*, yeast and wheat-germ polymerases B do not specifically transcribe the conalbumin, ovalbumin and adenovirus genes, in spite of the similarity between the A+T-rich presumptive promoter sequences in higher and lower eukaryotes, is perhaps not surprising in view of the low immunological cross-reactivity between the calf thymus RNA polymerase B on the one hand, and *Drosophila*³⁷ and yeast (A. Sentenac, personal communication) RNA polymerases B on the other, whereas immunological relatedness was observed for polymerases B of higher eukaryotes³⁸.

Although it is clear that cloned DNA can be transcribed specifically, the overall low efficiency of the *in vitro* system raises the question of whether some cellular post-synthetic DNA modifications (ref. 39 and refs therein) is required. Minor gene rearrangement of the ovalbumin gene during differentiation does not seem to be responsible for its low level of *in vitro* transcription, as the same results were obtained with DNA cloned from erythrocyte or oviduct cells (unpublished observation).

From all of these considerations and also the result of the experiment in which the conalbumin and ovalbumin templates were both present in the same incubation mixture (Fig. 4), we conclude that the factor(s) present in the S100 extract probably has an essential, but not exclusive, role in the initiation of transcription of most, if not all, messenger coding genes in higher eukaryotes. Several lines of evidence indicate that other important components involved in the *in vivo* regulation of transcription are missing from the present cell-free system. First, the major late adenovirus-2 gene is better transcribed *in vitro* than the early E1A gene, whereas early in the lytic cycle the major late gene is apparently not transcribed (see ref. 40 and refs therein). Second, the *in vivo* rate of transcription of the ovalbumin gene, under hormonal stimulation, can be at least as high as that of the conalbumin gene (see ref. 4 and refs therein), whereas it is obviously much lower *in vitro*. Third, the ovalbumin gene and the related X gene are similarly transcribed *in vitro*, whereas *in vivo* under hormonal stimulation the rate of transcription of the ovalbumin gene is at least 50 times higher than that of the X gene (ref. 20, and M. LeMeur and R. Palmiter, personal communications). The availability of an *in vitro* cell-free system which catalyses specific transcription of cellular genes provides a means of testing whether and how steroid hormone receptor complexes are involved in regulation of transcription, and represents the first step towards a complete elucidation of the molecular mechanisms involved in regulation of transcription in eukaryotic cells.

We thank Drs E. Bautz, A. Buchwalder, H. Faulstich, G. Krebs, A. Sentenac and H. Stein for gifts of materials, M. Cochet and F. Gannon for help with the cloning, J. M. Bornert, C. Hauss and C. Wasylyk for technical assistance, and K. Dott and L. Thia-Toong for growing cells and viruses. This investigation was supported by grants from the CNRS (ATP 3907, ATP 3558, ATP 4160), the INSERM (ATP 72.79.104) and the Fondation pour la Recherche Médicale Française.

Received 28 January; accepted 27 March 1980.

1. Losick, R. & Chamberlin, M. (eds) *RNA Polymerase* (Cold Spring Harbor Laboratory, New York, 1976).
2. Rosenberg, M. & Court, D. A. *Rev. Genet.* **13**, 319-353 (1979).
3. Chambon, P. *Cold Spring Harb. Symp. quant. Biol.* **42**, 1209-1234 (1978).
4. McKnight, G. S. & Palmiter, R. D. *J. biol. Chem.* **254**, 9050-9058 (1979).
5. Chambon, P. A. *Rev. Biochem.* **44**, 613-638 (1975).
6. Roeder, R. G. in *RNA Polymerase* (eds Losick, R. & Chamberlin, M.) 285-329 (Cold Spring Harbor Laboratory, New York, 1976).
7. Wu, G. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2175-2179 (1978).
8. Weil, P. A., Segall, J., Harris, B., Ng, S. Y. & Roeder, R. G. *J. biol. Chem.* **254**, 6163-6173 (1979).
9. Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. *Cell* **18**, 469-484 (1979).
10. Proudfoot, N. J. *Nature* **279**, 376 (1979).
11. Gannon, F. et al. *Nature* **278**, 428-434 (1979).
12. Cochet, M. et al. *Nature* **282**, 567-574 (1979).
13. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8**, 127-142 (1980).
14. Wu, G. J. & Zubay, G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1803-1807 (1974).
15. Kédinger, C. & Chambon, P. *Eur. J. Biochem.* **28**, 283-290 (1972).
16. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
17. Pericaudet, M., Akusjärvi, G., Virtanen, A. & Pettersson, U. *Nature* **281**, 694-696 (1979).
18. Ziff, E. B. & Evans, R. M. *Cell* **15**, 1463-1475 (1978).
19. Van Ormondt, H., Maat, J., De Waard, A. & Van der Eb, A. J. *Gene* **4**, 309-328 (1978).
20. Royal, A. et al. *Nature* **279**, 125-132 (1979).
21. Goldberg, M. thesis, Stanford Univ. (1979).
22. Smith, M. et al. *Cell* **16**, 753-761 (1979).
23. Krebs, G. & Chambon, P. *Eur. J. Biochem.* **61**, 15-25 (1976).
24. Greenleaf, A. L. & Bautz, E. K. F. *Eur. J. Biochem.* **60**, 169-179 (1975).
25. Sawadogo, M., Sentenac, A. & Fromageot, P. *J. biol. Chem.* (in the press).
26. Jendrisak, J. J. & Burgess, R. R. *Biochemistry* **14**, 4639-4645 (1975).
27. Dezébec, S. & Sentenac, A. *Eur. J. Biochem.* **34**, 41-52 (1973).
28. Gissinger, F. & Chambon, P. *Eur. J. Biochem.* **28**, 277-282 (1972).

29. Cochet-Meilhac, M., Nuret, P., Courvalin, J. C. & Chambon, P. *Biochim. biophys. Acta* **353**, 185–192 (1974).
30. Baker, C. C., Herisse, J., Courtois, G., Galibert, F. & Ziff, E. *Cell* **18**, 569–580 (1979).
31. McKay, R. *Nature* **282**, 556 (1979).
32. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. *Cell* **19**, 13–25 (1980).
33. Bogenhagen, D. F., Sakonju, S. & Brown, D. D. *Cell* **19**, 27–35 (1980).
34. Thimmappaya, B., Jones, N. & Shenk, T. *Cell* **18**, 947–954 (1979).
35. Kressmann, A., Holstetter, H., Di Capua, E., Grosschedl, R. & Birnstiel, M. *Nucleic Acids Res.* **7**, 1749–1763 (1979).
36. Stein, H. & Hausen, P. *Cold Spring Harb. Symp. quant. Biol.* **35**, 709–717 (1970).
37. Greenleaf, A. L., Krämer, A. & Bautz, E. K. F. in *RNA Polymerase* (eds Losick, R. & Chamberlin, M.) 793–801 (Cold Spring Harbor Laboratory, New York, 1976).
38. Ingles, C. J. *Biochem. biophys. Res. Commun.* **55**, 364–371 (1973).
39. Mandel, J. L. & Chambon, P. *Nucleic Acids Res.* **7**, 2081–2103 (1979).
40. Flint, J. *Cell* **10**, 153–166 (1977).
41. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* (in the press).
42. Suttcliffe, J. G. *Nucleic Acids Res.* **5**, 2721–2728 (1978).
43. Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
44. Brison, O. & Chambon, P. *Analyt. Biochem.* **75**, 402–409 (1976).
45. Wasyluk, B., Thevinin, G., Oudet, P. & Chambon, P. *J. molec. Biol.* **128**, 411–440 (1979).

Satellite tobacco necrosis virus structure at 4.0 Å resolution

T. Unge, L. Liljas, B. Strandberg, I. Vaara,
K. K. Kannan* & K. Fridborg

Department of Molecular Biology, University of Uppsala, Box 562, S-751 22 Uppsala, Sweden

C. E. Nordman & P. J. Lentz Jr†

Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

An electron density map of satellite tobacco necrosis virus at 4.0 Å resolution was obtained using single isomorphous replacement and phase refinement by icosahedral averaging. Near the N-terminus the polypeptide chain is a three-turn helix, in contact with 3-fold related helices, extending towards the particle centre. The protein subunit has at least one twisted β -sheet.

THE satellite tobacco necrosis virus (STNV) is one of the smallest known viruses. It requires *in vivo* co-infection with tobacco necrosis virus for growth^{1,2}. STNV is composed of a coat of 60 identical protein molecules (molecular weight (MW) 21,600^{3,4}) and a single-stranded RNA, known to code for the coat protein⁵. The complete sequences of both STNV RNA³ (about 1,200 nucleotides) and the STNV protein^{3,4} (195 amino acids) are known. The coat protein is arranged in a $T=1$ icosahedral surface lattice^{6–8}. Thus, all 60 protein subunits have identical surroundings in the virus particle.

STNV crystallises from 1.0×10^{-3} M MgSO_4 in space group C2 with four particles in the monoclinic cell with $a=318.4$ Å, $b=305.0$ Å, $c=185.3$ Å and $\beta=94.37^\circ$. The crystallographic asymmetric unit is a complete virus particle⁹, with an MW of 1.7×10^6 (refs 3, 10, 11). That is, the virus particle is located in a general position in the crystallographic unit cell. Hence, the crystallographic symmetry does not exclude a determination of even the nucleic acid structure.

This article describes the structure of STNV at 4.0 Å resolution. The electron density maps were calculated with phases obtained by two procedures, in both cases using double isomorphous replacement (DIR) phases at 10 Å resolution¹² as starting phases. In one method the phases in the 10–4 Å resolution range were conventional single isomorphous replacement (SIR) phases, refined with a symmetry averaging (SA) technique, which makes use of the redundancy in the crystal data due to the 60-fold non-crystallographic symmetry of the virus protein. In the other method a combined phase extension and phase refinement was done in small steps from 10 to 4 Å resolution by the SA technique.

The preparation of STNV crystals⁹, the preparation of the PtCl_4^{2-} and iodine derivatives used at 10 Å resolution, and the location and refinement of these heavy atom sites have been described elsewhere¹². A modified iodine derivative with two iodine positions per subunit was prepared¹³ and used for SIR at 4.0 Å resolution. The 4 Å X-ray diffraction data from native STNV crystals and STNV iodine crystals were collected using oscillation camera technique as described in Table 1. The statistics of the refinement of the two iodine atoms are given in Table 2.

Electron density calculation based on SIR and SA

The DIR-phases at 10 Å resolution were taken as the initial values for phase refinement by means of non-crystallographic symmetry^{14–16}. After three cycles of refinement at 10 Å (with wF_{obs} as the structure amplitude; see Table 3) an electron density map was computed using the refined phases to 10 Å and 'best'¹⁷ SIR phases from 10 to 4 Å resolution. This map was the starting point for eight cycles of refinement of the phases using symmetry averaging (see Table 3). No combination of SIR and SA phases was done. The electron density in each refinement cycle was calculated with a grid spacing of 1.9 Å, and 60-fold icosahedral averaging of the electron density was performed using a grid sampled at 1.7 Å intervals within a region limited by an outer, and in the four last cycles also by an inner, envelope¹⁸. The outer envelope was a sphere of 88 Å radius modified by addition of smaller spheres close to the 5-fold axes to account for protrusions of continuous density to a maximum radius of 96 Å. The inner envelope was a sphere of 62.5 Å radius with addition of truncated cones around the 3-fold axes penetrating to 43 Å particle radius, accounting for strong continuous density. Outside the outer envelope the electron density was levelled. Inside the inner envelope the electron density values were

*Present address: Neutron Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.

†Present address: Department of Biology, King's College, Wilkes-Barre, Pennsylvania.

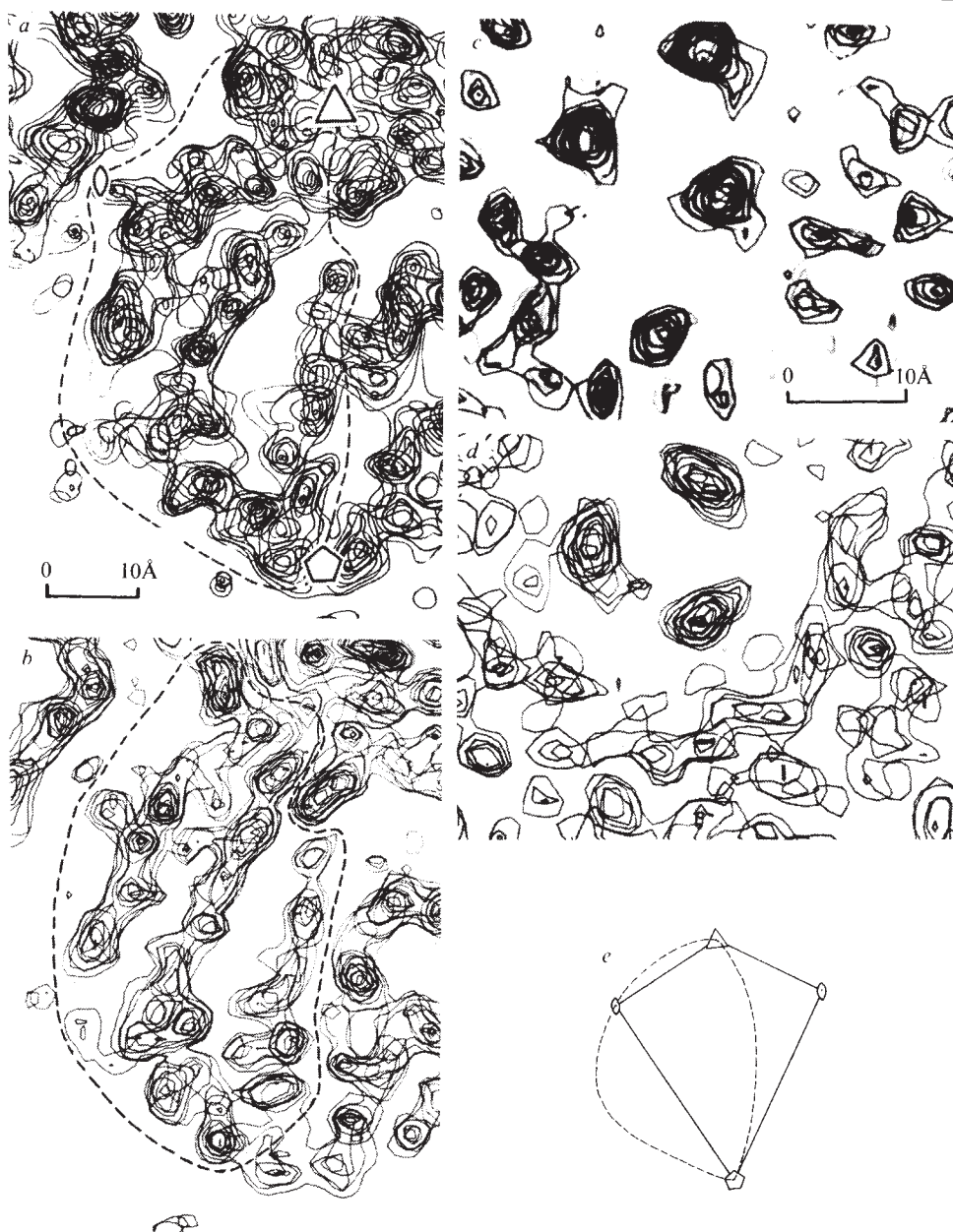


Fig. 1 Comparison of electron density maps obtained by SIR+SA (a and c) and PE+SA (b and d). All sections are plotted perpendicular to one particle 3-fold axis. Subunit boundaries are indicated. Parts a and b compare sections at 79–76 Å from the particle centre showing the protein subunit region. The densities in the PE are better resolved than the densities in the SIR maps, indicating that the SIR maps suffer from incomplete phase information. Parts c and d show the sections at 57–54 Å particle radii containing the densities interpreted as part of the 3-fold-related helices anchoring three subunits to each other. e, Key to Figs 1 a–d, 2a, b and 3a, b showing the locations of the particle 3-fold axis (perpendicular to paper), 5-fold and 2-fold axes (tilted), the boundaries of one sixtieth, that is one asymmetric unit of the virus particle (solid line), and indicating approximate subunit boundaries (broken line).

essentially left unmodified; a damping function was used to prevent build-up of noise¹⁸. In the phase refinement the F_{calc} values from the previously averaged map were put in for unrecorded reflections.

Electron density calculation based on phase extension (PE) and SA

The 4 Å PE calculation was based on 10 Å DIR-phases, refined with SA (three cycles). Phases in the resolution range 10–4 Å were calculated in a stepwise phase extension procedure. The resolution was increased in small increments: 2,000–3,500 reflections were added in each step. The F_{calc} values in the extended range were calculated from the latest electron density map, based on F_{obs} amplitudes and $\alpha_{\text{SA calc}}$ phases at somewhat lower resolution. The detailed procedure is explained in ref. 18. The total phase extension from 10 to 4 Å resolution included about 50 cycles of phase refinement. Grid spacing, choice of envelope and other conditions were as for the refinement of the SIR map, with the exception that no weighting factor was applied to the $|F_{\text{obs}}|$ values in the PE+SA calculation.

Argos *et al.*¹⁹ used a phase extension procedure from 6.0 to

4.9 Å resolution in their crystallographic studies of glyceraldehyde-3-phosphate dehydrogenase (GPD) with two 2-fold non-crystallographic symmetry axes. The experience from their study was the necessity to use a rather accurate molecular envelope and the need for small increments in data resolution. Also, in the present STNV studies we have noticed the importance of good molecular envelopes (in the case of a spherical virus this is fairly simply determined) and have, in order to properly use the coupling between phase angles, also chosen small data resolution increments.

Comparison of SIR+SA and PE+SA electron density maps

Sections of the SIR+SA and PE+SA electron density maps are shown in Fig. 1. There is good agreement between the maps in the region corresponding to the protein part. In the SIR+SA map the continuity is more pronounced, while the PE map contains somewhat more resolved densities. The dissimilarities might well be explained by the rather inaccurate starting SIR phases (the main reason for this being one heavy atom derivative; other factors are incomplete data and limited

refinement of the heavy atom parameters), together with the rather limited number of refinement cycles in the SIR-SA calculation as well as differences in weighting between the methods.

Structure results

The final STNV structure, shown in Figs 2–4, is based on the PE+SA calculation at 4 Å resolution followed by several phase refinement cycles in which the Fourier and averaging grid spacings were 1.3 and 1.05 Å respectively, and the data resolution was increased to nominally 3.8 Å (about half of the F_{obs} values in the region 3.8–4.0 Å were available). A total of 135,808 reflections out of the 168,180 possible reflections were observed. The R factor for the observed reflections was 21%.

General distribution of electron density in the STNV particle

The minimum particle radius is about 86 Å in the 3-fold direction, and the maximum is 96 Å in the 5-fold direction, roughly giving the particle the shape of a regular icosahedron. The inner boundary for the main protein part has a mean radius of about 65 Å. At this radius there is a thin layer of low electron density dividing the main protein part from the interior of the particle (Fig. 3c). Also the electron density distribution of southern bean mosaic virus (SBMV)²⁰ contains a similar 'valley' between the protein part and the interior of the particle. The density in the interior of the particle probably reflects the distribution of the RNA. The mean radial distribution of RNA and protein is in good agreement with results from small angle neutron scattering¹¹. In the vicinity of the 5-fold axes the STNV RNA structure extends significantly beyond the 65 Å radius, and an apparent 'stacking' of electron density at 60–75 Å radius suggests an at least partially ordered RNA structure in these regions (Fig. 3d). The present map has not allowed a simple interpretation of the nucleic acid. Whether this is due to a statistically distributed or disordered RNA or to limitations in the phase determination remains to be settled.

Structure of the STNV protein subunit

The subunit boundaries are obvious except for a few places near the 3-fold and 5-fold axes (Fig. 2a, b). The subunit consists of one main domain, with an overall size of 25 Å × 30 Å × 50 Å, located between particle radii 60 and 96 Å and

Table 1 Statistics of data collection

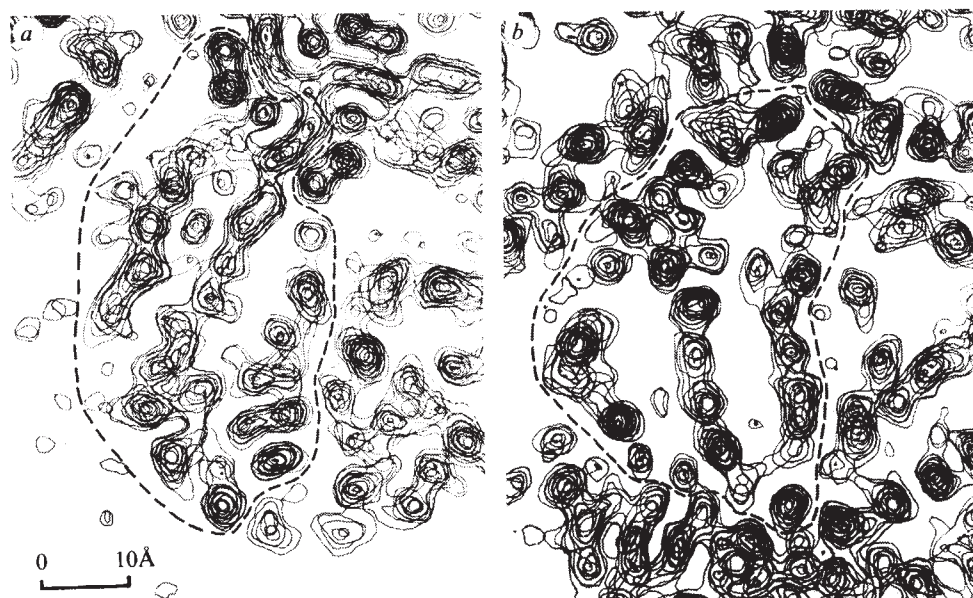
4 Å data*		
	Native	STNV-iodine derivative
Fully recorded reflections measured	169,000	145,000
No. of independent reflections measured of 148,100 possible	117,000	105,000
$R_{\text{sym}} = \sum_h \left(\sum_i F_{hi} - \bar{F}_h \right) / \sum_{h,i} F_{hi} $	0.067	0.090
Reflections used in the phase calculation†		
Compound	Resolution	No. of reflections in the phase calculation
STNV-PtCl ₄ ²⁻	10 Å	7,300
STNV-iodine	10 Å	7,400
STNV-iodine	4 Å	78,300

Data were collected using a Nonius oscillation camera and an Elliott rotating anode generator equipped with a graphite monochromator or focusing mirrors. The 4-Å oscillation photographs were taken with a 1.2° oscillation range. The crystals were rotated about the c^ axis with a total oscillation range of 90°. The exposure time was 3–4 h per film set. Up to eight exposures were obtained from one position on a crystal. The films were measured with an Optronics film scanner (50 µm raster size), and the data were processed with the film processing program of Schwager *et al.*²⁵. All partial reflections and reflections with integrated intensities below background were rejected before scaling²⁶. The 4-Å native data set was merged with a 5.5-Å precession camera data set giving 124,395 observed reflections out of the 148,100 possible reflections.

†A value for B_{overall} of zero [$F_{\text{Der}} = K F_{\text{Nat}} \exp(-B_{\text{overall}}(\sin \theta/\lambda)^2)$] for the 4.0 Å STNV-iodine data was obtained from three-dimensional heavy atom refinement. For the 10 Å data a value for $B_{\text{overall}} = 0$ was used without refinement. All reflections which were registered on both the native and corresponding heavy atom derivative data set were used in the phase determination.

an arm extending from the main domain into the particle at least to 47 Å radius (Fig. 4, Fig. 1d). The continuity of density is good within the whole subunit and there is one large, twisted, probably three-stranded sheet (Fig. 2a, b). In some parts of the main domain the polypeptide chain can be traced. The central portion of the arm has the approximate dimensions of a three-turn α -helix, is positioned close to the 3-

Fig. 2 Composite electron density sections (PE, 1.3-Å map grid spacing, 3.8 Å data resolution; see text) perpendicular to a particle 3-fold axis at a, 80–77 Å and b, 72–69 Å from the particle centre. Within the marked protein subunit there are ribbons of continuous electron density, whose directions show a left-handed change in going from a and b in agreement with the twist in β -sheets.



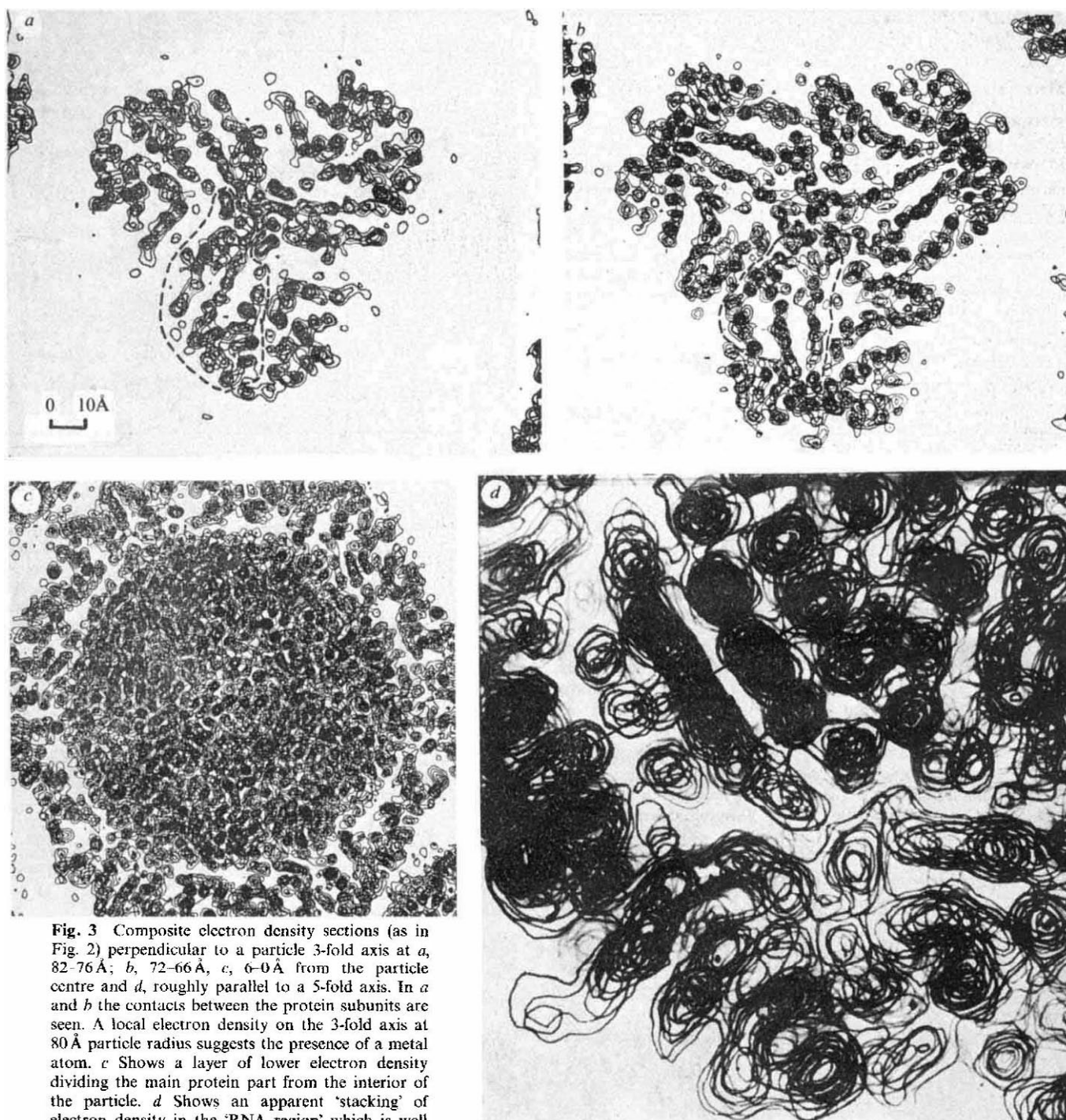


Fig. 3 Composite electron density sections (as in Fig. 2) perpendicular to a particle 3-fold axis at *a*, 82–76 Å; *b*, 72–66 Å, *c*, 6–0 Å from the particle centre and *d*, roughly parallel to a 5-fold axis. In *a* and *b* the contacts between the protein subunits are seen. A local electron density on the 3-fold axis at 80 Å particle radius suggests the presence of a metal atom. *c* Shows a layer of lower electron density dividing the main protein part from the interior of the particle. *d* Shows an apparent 'stacking' of electron density in the 'RNA region' which is well separated from the protein part (to the left in the picture). The apparent 'RNA layers' are positioned almost perpendicular to a particle 5-fold axis (arrow in the plane of the paper), at 60–75 Å particle radius and with 5.5 Å spacing between the 'RNA layers'.

fold particle axis, and is in contact with the arms of the two neighbouring 3-fold related subunits. The helices are tilted about 45° relative to each other. The figures have been drawn with a choice of hand which is based on the following observations: (1) The electron density maps shown (Fig. 2*a, b*) contain at least one β -pleated sheet with a left-handed twist. (2) Inspection of the electron density corresponding to the previously mentioned three-turn α -helix as well as another tentative helix indicates right-handedness. This choice of hand gives a right-handed supercoil of the three helices at a 3-fold particle axis.

The sequence of STNV RNA and the STNV protein have recently been determined^{3,4}. In the iodine derivative¹³ an average of 1.5 iodine atoms bind to the same histidine residue close to the N-terminus (His 23). The iodine atoms are

positioned on the long supercoiled arms, at particle radii of 56 and 59 Å respectively (Fig. 4*a*). From the knowledge of the amino acid sequence^{3,4} it has been predicted that this part of the polypeptide chain is, to a high probability, in the form of an α -helix^{21,22}. Of the 30 amino acids at the N-terminal (which include the arm) nine are basic residues and only one acidic^{3,4}, suggesting a strong interaction between this part of the protein and the nucleic acid. Model building of the α -helical region (residues 17–27), containing residue His 23 (with the marker iodine atoms), can be done placing all the four basic residues on the outside and the hydrophobic residues on the inside of the supercoil. The platinum position in the 10 Å STNV-Pt derivative is also on the outer surface of the helix at a particle radius of 51 Å. Positioning the PtCl_4^{2-} group in the above mentioned model suggests attachment to amino acid

Table 2 Heavy atom parameters

Compound	Resolution	Site	Z	B	x	y	z	R _{mod}
STNV-PtCl ₄ ²⁻	10 Å	Pt	55	120	0.3131	0.4063	0.3004	0.098
r.m.s. f _c /r.m.s. E = 2.2, <ΔF>/<F _{Der} > = 0.10; R _c = 0.32								
STNV-iodine	10 Å	I	32	85	0.3321	0.4277	0.2931	0.098
r.m.s. f _c /r.m.s. E = 1.3, <ΔF>/<F _{Der} > = 0.10; R _c = 0.60								
STNV-iodine	4 Å	I ₁	42	7	0.3303	0.4287	0.2913	0.174
		I ₂	41	6	0.3247	0.4166	0.2929	
r.m.s. f _c /r.m.s. E = 1.24 (1.41 and 1.20 for low and high sin θ/λ values, respectively)								
<ΔF>/<F _{Der} > = 0.17; R _c = 0.75								

Z, occupancy in electrons on an approximate absolute scale.

B, isotropic thermal parameter for the heavy atoms.

$$R_{\text{mod}} = \frac{\sum_h |F_{\text{Der, obs}}| - |F_{\text{Der, calc}}|}{\sum_h |F_{\text{Der, obs}}|}$$

$$R_c = \frac{\sum_{h0l \text{ refl.}} |\Delta F_{\text{obs}}| - |\Delta F_{\text{d}}|}{\sum_{h0l \text{ ref}} |\Delta F_{\text{obs}}|}$$

E, lack of closure at best phase angle.

f_c, heavy atom structure factor.

Figures of merit: DIR (10 Å) = 0.62 SIR (4 Å) = 0.34.

The parameters corresponding to the 10 Å data were refined by structure factor fitting using *h0l* reflections only¹². The x, y, z parameters for position I₁ and I₂ were determined from a difference Fourier calculation using DIR phases (>10 Å resolution) and PE phases (10–5.5 Å resolution) refined by SA. The positional parameters were confirmed by a difference Fourier calculation using 10 Å Pt phases but not further refined. Scale factor for the 4 Å iodine-derivative and occupancies for I₁ and I₂ were refined by three-dimensional least squares technique. The occupancy for each of the 2 × 60 sites were refined separately and the occupancies were 60-fold averaged between each cycle.

Met 19. About the first 15 amino acids at the amino end cannot be traced in the map. The general arrangement of the subunits, showing the subunit contacts, is given in Fig. 3.

In the three spherical plant viruses which have been extensively studied by X-ray crystallography we can see similarities in the arrangement of the amino terminal end. The N-terminal part of the C subunits of tomato bushy stunt virus (TBSV)²³ and SBMV²⁴ as well as of STNV points into the particle close to the 3-fold axis, and is partly flexible.

We thank Dr A. Liljas for discussions, and Dr P. Argos, Professor W. Fiers, Dr D. Henriksson, Professor M. G. Rossmann, Professor R. E. Tashian and their colleagues for permission to refer to their unpublished results. We also thank Mrs M. Petef for help in data collection, Mrs A. Borell for programming assistance, Mr S. Lövgren for assistance with photography and Mrs G. Johansson for growing the virus and typing the manuscript. This was supported by grants from the

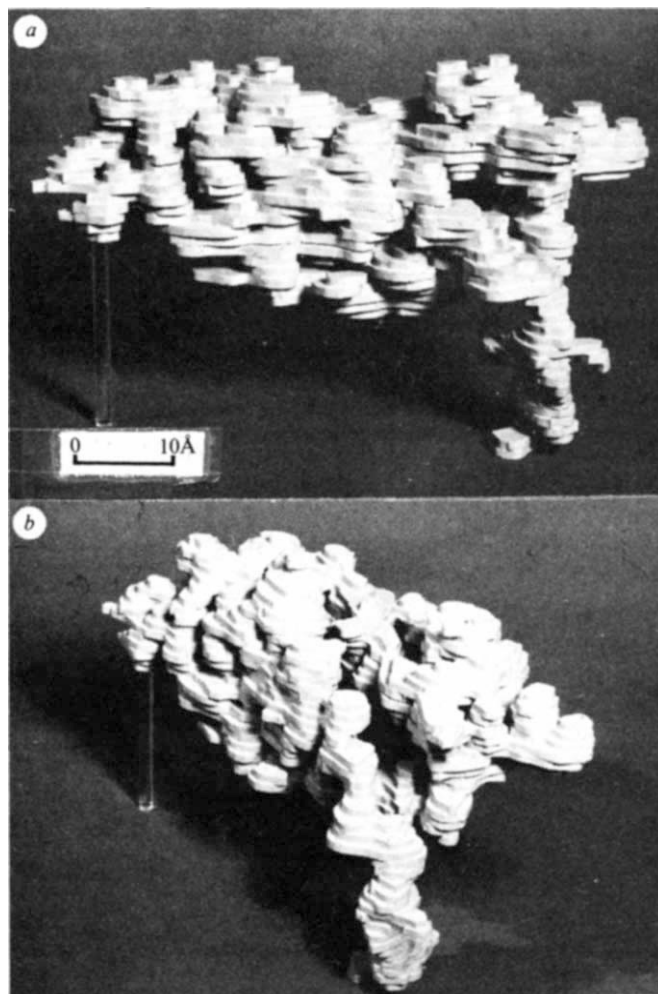


Fig. 4 Photographs of a model of the protein subunit. *a*, Viewed approximately perpendicular to the particle 3-fold axis showing the α-helical arm which extends toward the particle centre. The two iodine atoms bound to His 23 are marked. *b*, View of the outer part of the protein subunit showing structural features described in Fig. 2.

Faculty of Science, Uppsala University, the Swedish Natural Science Research Council (K 2142), the Magnus Bergvall Foundation, the E. and K. G. Lennander Foundation, the Knut and Alice Wallenberg Foundation and the US NIH (GM 15259).

Received 4 January; accepted 5 April 1980.

- Kassanis, B. & Nixon, H. L. *J. gen. Microbiol.* **25**, 469–471 (1961).
- Rees, M. W., Short, M. N. & Kassanis, B. *Virology* **40**, 448–461 (1970).
- Ysebaert, M., van Emmelo, J. & Fiers, W. *J. molec. Biol.* (submitted).
- Henriksson, D., Tanis, R. J., Hawett-Emmett, D., Tashian, R. E. & Nyman, P. O. *J. molec. Biol.* (submitted).
- Reichmann, M. E. *Proc. natn. Acad. Sci. U.S.A.* **52**, 1009–1017 (1964).
- Åkervall, K. *et al. Cold Spring Harb. Symp. quant. Biol.* **36**, 469–483; 487–488 (1971).
- Klug, A. *Cold Spring Harbor Symp. quant. Biol.* **36**, 483–487 (1971).
- Lentz Jr., P. J. & Strandberg, B. *Acta crystallogr. A* **30**, 552–559 (1974).
- Fridborg, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **54**, 513–521 (1965).
- Sjöberg, B. *Eur. J. Biochem.* **81**, 277–283 (1977).
- Chauvin, C., Jacrot, B. & Witz, J. *Virology* **83**, 479–481 (1977).
- Lentz, P. J. Jr. *et al. Acta crystallogr. B* **32**, 2979–2983 (1976).
- Unge, T. & Strandberg, B. *Virology* **96**, 80–87 (1979).
- Rossmann, M. G. & Blow, D. M. *Acta crystallogr.* **16**, 39–45 (1963).
- Bricogne, G. *Acta crystallogr. A* **30**, 395–405 (1974).
- Bricogne, G. *Acta crystallogr. A* **32**, 832–847 (1976).
- Blow, D. M. & Crick, F. H. C. *Acta crystallogr.* **12**, 794–802 (1959).
- Nordman, C. E. *Acta crystallogr.* (in the press).
- Argos, P., Ford, G. C. & Rossmann, M. G. *Acta crystallogr. A* **31**, 499–506 (1975).
- Suck, D., Rayment, I., Johnson, J. E. & Rossmann, M. G. *Virology* **85**, 187–197 (1978).
- Unge, T. thesis (no. 523), Uppsala Univ. Faculty of Science (1979).
- Argos, P. in *Proc. 7th Aharon Katzir-Katchalsky Conf. on Structural Aspects of Recognition and Assembly in Biological Macromolecules*, Nof Ginosor, Israel (in the press).
- Harrison, S. C., Olson, A. J., Schutt, C. E., Winkler, F. K. & Bricogne, G. *Nature* **276**, 368–373 (1978).
- Abad-Zapatero, C. *et al. Nature* (in the press).
- Schwager, P., Bartels, K. & Jones, A. J. *appl. Crystallogr.* **8**, 275–280 (1975).
- Hamilton, W. C., Rollett, J. S. & Sparks, R. A. *Acta crystallogr.* **18**, 129–130 (1965).

Table 3 Phase refinement of SIR phases by non-crystallographic symmetry (MR)

Cycle no. at 4 Å resolution	<w>*	<Δα>†	R‡
1	0.34§	51.0	0.63
2	0.49	20.8	0.47
3	0.65	16.6	0.40
4	0.70	12.7	0.37
5	0.73	10.4	0.36
6	0.83	9.0	0.34
7	0.84	9.6	0.32
8	0.84	7.0	0.32

*Mean weighting factor; the weighting factor was a function of $F_{\text{obs}}/F_{\text{calc}}$ for individual reflections.

†Mean phase change (in degrees) for all reflections; the total average phase change was 62°.

$$\ddagger R = \frac{\sum_h |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum_h |F_{\text{obs}}|}$$

where F_{obs} is the measured amplitude and F_{calc} is the amplitude calculated from an icosahedrally averaged map.

§Figure of merit for the SIR phases.

Analysis and prediction of protein β -sheet structures by a combinatorial approach

Fred E. Cohen, Michael J. E. Sternberg & William R. Taylor

Laboratory of Molecular Biophysics, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

Analysis of β -sheet sandwiches (for example immunoglobulin domains) suggests an algorithm that successfully predicts the tertiary fold of these proteins from their sequence and secondary structure. We propose tertiary structures for β_2 -microglobulin and an HLA-B7 antigen fragment. Topological rules are presented that markedly reduce the number of folds for proteins in which α -helices pack against a parallel β -sheet.

WITH the structures of more than 60 globular proteins known to atomic resolution, it is clear that the tertiary fold is largely determined by the packing of α -helices and/or β -strands¹⁻⁷. For many proteins¹, their fold can adequately be described by one of three motifs: a stacked pair of β -sheets (β/β); α -helices packing against a predominantly parallel β -sheet (α/β); or an assembly of α -helices (α/α). These arrangements satisfy the hydrogen-bonding requirements of buried main-chain nitrogen and oxygen atoms while shielding a substantial fraction of the nonpolar atoms from solvent².

From these observations we are developing a stepwise method of predicting the three-dimensional structure of a protein from its amino acid sequence (for reviews see refs 8-10). We envisage three stages: (1) predict the regular secondary structures, now possible with up to 80% accuracy; (2) pack the α -helices and β -strands into an approximate native fold, (3) use simplified energy calculations¹¹⁻¹³ to refine the fold into the native structure.

This approach is timely as recent work^{14,15} has shown that the structures predicted solely by simplified energy calculations are not significantly better than random models for a compact globular protein. This article considers the second stage which is implemented by a combinatorial method. Initially one generates a list of trial structures by packing all combinations of the α -helices and β -strands. The native fold will be in this list and so one simply eliminates structures that violate

stereochemical rules. This approach has been applied to the packing of α -helices in myoglobin^{7,16,17}. Over 10^8 trial structures were generated by docking together predicted hydrophobic patches on the surfaces of the α -helices. However, only 20 folds did not violate the steric and connectivity constraints. The addition of two distance constraints obtained from experimental data on haem binding further restricted the number of allowed structures to two. The relative arrangements of α -helices in one of these structures closely resembled that in the native structure. The root mean square deviation^{15,18} between this predicted structure and the native (r.m.s.d.) was 4.3 Å.

The success of this approach motivated our analyses of structures formed from β -sheet sandwiches or from α -helices packing against parallel β -sheets. This led to combinatorial algorithms to predict allowed tertiary folds.

Analysis of β -sheet sandwiches

The stability of a β -sheet sandwich results from inter-strand hydrogen bonding and the burial of hydrophobic residues both within and between the β -sheets. We estimated the contribution of the hydrophobic effect to the free energy of folding from the change in the solvent accessible contact area^{7,19} of the nonpolar (C^α , side chain C and S) atoms (Φ -area) by the empirical relationship $1 \text{ Å}^2 \equiv 80 \text{ cal mol}^{-1}$ (ref. 7).

The structures of nine β -sheet sandwiches (Fig. 1) were

Table 1 Prediction of β -sheet sandwiches

Structure*	No. of strands†	No. of possible structures from all strands‡	No. of structures generated from strands with sites§	Position in list of strand overlap of native structure	r.m.s.d. predicted/crystal structure¶ (Å)	No. of residues
FCH2	7(6)	5×10^7	6×10^5	327 (154)	2.3	34
FCH3	7(7)	5×10^7	3×10^6	36 (9)	4.9	50
FALC	7(7)	5×10^7	3×10^6	10 (5)	1.4	46
FAHC	7(7)	5×10^7	3×10^6	6 (6)	3.2	48
PRE	8(7)	2×10^8	3×10^6	12 (10)	2.0	51
FALV	8(8)	2×10^8	1×10^7	3,343 (494)	4.9	48
SDM	8(8)	2×10^8	1×10^7	1,135 (280)	5.1	51
FAHV	9(7)	6×10^8	3×10^6	509 (105)	3.5	46
REI	9(7)	6×10^8	3×10^6	988 (165)	3.6	48
B2-M	7(6)	5×10^7	6×10^5	34 (6)	—	53
AC-2	7(6)	5×10^7	6×10^5	93 (5)	—	48

*See Fig. 1 for abbreviations.

†The no. of β -strands in the sandwich with the no. of strands with sites in brackets.

‡For n strands, the number of topologies for two 3-stranded sheets is $(nC_3)(nC_3) 3!12^3 2^3/4$, where $(nC_r) = n!/(n-r)!r!$. For each topology we estimate that there are 625 (that is, 5^4) alternative sheet structures that have a high degree of strand overlap.

§A lower estimate of the number of sheet structures generated from strands with sites placed with the 32 different phasings. In the top sheet the central residues can be phased in six ways: $-2, 0, +2$; $-2, 0, 0$; $0, 0, +2$; $0, 0, 0$; $+2, 0, 0$; $0, 0, -2$. In the bottom sheet the six phasings are: $+2, 0, -2$; $+2, 0, 0$; $0, 0, -2$; $0, 0, 0$; $-2, 0, 0$; $0, 0, +2$. Every pairing is allowed except for either of the last two phasings in the top sheet with the last two in the bottom sheet as both these phasings have the wrong direction.

||The number of strand-alignment diagrams (with the number of different topologies in brackets) that have the same or higher overlap than the chosen approximation to the native.

¶The root mean square deviation between equivalenced C^α atoms in the native and predicted six-stranded core. Thus accurate prediction of the strand-alignment diagram is sufficient to yield a C^α structure close enough to the native that energy minimization could be applied.

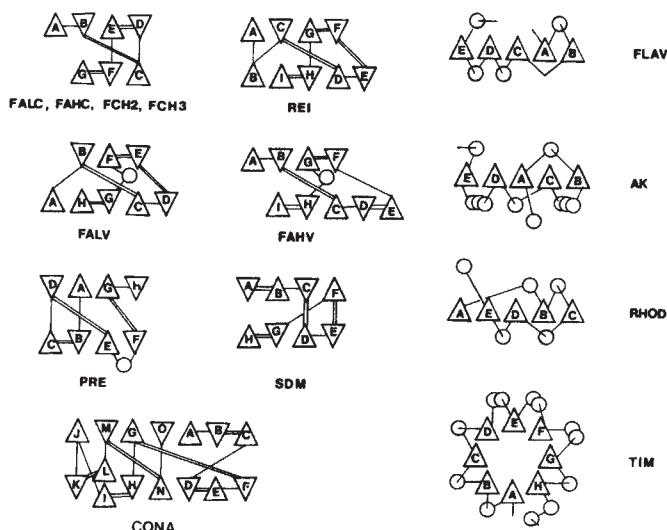


Fig. 1 The fold of β/β and α/β proteins: schematic diagrams of all the β -sheet sandwiches and some of the α -helix/ β -sheet proteins considered. Each β -strand is viewed along its strand direction and is represented by a triangle whose apex points up or down according to whether the strand is viewed from the N- or the C-terminus. A circle represents an α -helix. For the β -sheet sandwiches, the connections are shown in double lines if they start at the end of the sheet close to the viewer, otherwise in single lines. FAHV, FAHC, FALV, FALC—the heavy (H) and light (L) chains of the variable (V) and constant (C) domains of Fab' human immunoglobulin IgG(λ) new fragment²¹. FCH2, FCH3, C_{H2} and C_{H3} domains of human Fc (ref. 22). REI, Bence-Jones variable dimer. PRE, human prealbumin²³. SDM, Cu,Zn superoxide dismutase. CONA, jack bean concanavalin A. FLAV, *Clostridium* flavodoxin. AK, porcine adenylyl kinase. RHOD, bovine rhodanese (one domain). TIM, chicken triose phosphate isomerase. Coordinates were obtained from the Brookhaven Data Bank²⁹ except where a reference is given to the laboratory that supplied them.

dissected to find the patterns of changes in Φ -area in the transition from isolated secondary structure to the native conformation. Each β -strand was composed of residues that either formed intra-sheet hydrogen bonds or were in the main-chain conformation $-170^\circ < \phi < -90^\circ$, $100 < \psi < 170$. For each sandwich, three sets of Φ -areas were calculated from the crystallographic coordinates^{20–23}:

- (1) The sum of the Φ -areas of all the β -strands considered in isolation.
- (2) The sum of the Φ -areas of the two β -sheets considered individually.
- (3) The Φ -area of the stacked pair of β -sheets.

We can now assess the hydrophobic free energy change for the stepwise process of first condensing β -strands into two β -sheets followed by the stacking of the two sheets. For prealbumin, these values are -74 and -24 kcal mol⁻¹. These values are the differences of set 2 from set 1, and of set 3 from set 2, respectively. Similar hydrophobic interactions occur in all the other sheet sandwiches: the values of these two area changes divided by the number of residues in the two sheets are in the ranges -0.9 to -1.2 and -0.3 to -0.6 kcal mol⁻¹ per residue. Ancillary calculations on prealbumin demonstrate the stability of the sheet sandwich. The -98 kcal mol⁻¹ obtained in condensing the eight strands into the sandwich represent 54% of the total change in folding a hypothetical extended polypeptide chain into the native monomeric structure. The subsequent formation of the tetramer from the monomers yields an additional -21 kcal mol⁻¹ per subunit.

The specificity of the interactions is revealed by the Φ -area changes of the individual residues. Forming a β -sheet from isolated β -strands produces changes for most residues irrespective of their chemical nature. The effect of the twist of the β -sheet²⁴ is a reduction in the area change for the residues at the strand termini.

The strand alignment diagrams in Fig. 2 present the observed area changes on sheet stacking for several immunoglobulin domains. In a β -sheet sandwich, side chains in each β -strand point alternately towards and away from the other β -sheet, producing two surfaces on each sheet. Surprisingly, in these diagrams and in the other sandwiches only 2/3 of the residues in the 'buried' faces have substantial area change ($>5 \text{ \AA}^2$). This explains why, when immunoglobulin sequences are aligned²⁴, only some of the β -strand side chains that point towards the other sheet are always nonpolar.

The most important feature of Fig. 2 for our prediction algorithm is that the observed area changes trace a distinct pattern on the β -sheets. In the top sheet the residues with sizeable area changes progress from left to right, going down the diagram, whereas in the bottom sheet they proceed from right to left. This anti-complementary direction of the two parallelogram patterns results from the geometry of sheet packing. The twist²⁵ between adjacent β -strands forces the β -sheet roughly to resemble a deformed rectangle with diagonally opposite corners pointing either both up or both down (Fig. 3). Consequently the major hydrophobic interactions on sheet stacking are between the lower two corners of the top sheet and the upper corners of the bottom

Table 2 Allowed β -sheet topologies

Protein	No. of strands	No. of possible topologies	No. of topologies allowed by rule 1	No. of topologies allowed by rules 1–4
Flavodoxin	5	960	60	6
Adenyl kinase	5	960	60	6
Rhodanese				
first or second domain	5	960	60	6
Alcohol dehydrogenase				
NAD binding domain	6	11,520	360	15
Phosphoglycerate kinase				
catalytic domain	6	11,520	360	10
Phosphoglycerate kinase				
ATP domain	6	11,520	360	10
Lactate dehydrogenase				
NAD domain	6	11,520	360	13
Triose phosphate isomerase	8	5×10^6	40,320	21

The protein domains listed are formed from α -helices stacking against a pure parallel β -sheet. The total number of topologies for the β -strands and their connections is reduced by rule (1) and then by the use of rules (1) to (4).

sheet. This effect is enhanced by shortening some of the strands and removing residues not involved in sheet/sheet interactions⁵, for example in the immunoglobulin domain designated FAHV, the carboxyl end of strands A and F.

Algorithm to predict the structure of β -sheet sandwiches

We start with the sequence and an observed (or predicted) strand assignment. Each strand is considered as a unit and we aim to predict the correct strand-alignment diagram which can then be converted to C $^\alpha$ coordinates. The algorithm is outlined below. The first three steps generate all possible sandwiches and the fourth step removes disallowed folds. The computer program is available on request.

(1) *Location of a hydrophobic site on each β -strand.* First, one predicts not only which surface of each β -strand will point towards the other β -sheet but also the location of the few residues that mediate the hydrophobic interaction. Each strand was scanned for a continuous site of nonpolar residues on one of its sides, for example three residues at positions $i-2$, i , $i+2$. The central residue at position i represents this site. To distinguish between alternate central residues we consider first the number of nonpolar residues in the site (4, 3 and 2) and

FCH3 OBSERVED		AC-2 PREDICTED	
A +	arg <u>GLU</u> gln <u>VAL</u> <u>TYR</u> <u>THR</u> leu	A + -2	ASP <u>PRO</u> pro <u>LYS</u> thr HIS val
B +	tyr <u>PHE</u> lys <u>VAL</u> leu <u>CYS</u> thr <u>LEU</u> ser VAL gln	B + 0	tyr <u>PHE</u> leu <u>ALA</u> trp <u>CYS</u> arg <u>LEU</u> thr <u>ALA</u> glu
E +	ser <u>PHE</u> phe <u>LEU</u> tyr <u>SER</u> lys <u>LEU</u> thr <u>VAL</u>	E + +2	THR phe <u>GLU</u> lys <u>TRP</u> ala <u>ALA</u> val <u>VAL</u> val
D +	asp <u>LEU</u> val <u>PRO</u> thr <u>THR</u> lys <u>TYR</u> asn	D +	gly <u>ALA</u> arg <u>THR</u> glu <u>VAL</u> leu <u>GLU</u> thr
G +		G + 0	
F +	met <u>VAL</u> ser <u>CYS</u> ser <u>PHE</u> val	F + 0	<u>LEU</u> pro <u>LYS</u> pro <u>LEU</u> thr
C +	ala <u>VAL</u> glu <u>TRP</u> glu	C + -2	HIS gln <u>VAL</u> his <u>CYS</u> thr <u>TYR</u>
			<u>ILE</u> thr <u>LEU</u> thr <u>TRP</u> gln
FALC PREDICTED		B2-M PREDICTED	
A + 0	ser <u>VAL</u> thr <u>LEU</u> phe <u>PRO</u>	A + -2	ile <u>GLN</u> arg <u>THR</u> lys <u>ILE</u> gln <u>VAL</u> tyr <u>SER</u> arg
B + 0/+2	ASP ser <u>ILE</u> leu <u>CYS</u> val <u>LEU</u> thr <u>ALA</u>	B + 0	his <u>PHE</u> ser <u>VAL</u> tyr <u>CYS</u> asn <u>LEU</u> phe
E + +2	lys <u>TYR</u> ala <u>ALA</u> ser <u>SER</u> tyr <u>LEU</u> ser <u>LEU</u> thr	E + 0	ser <u>PHE</u> tyr <u>LEU</u> leu <u>TYR</u> ser <u>TYR</u> thr
D +	gln <u>LYS</u> ser <u>PRO</u> thr <u>THR</u> glu <u>VAL</u> gly	D +	glu <u>VAL</u> lys
G + 0	SER thr <u>VAL</u> glu <u>LYS</u> thr <u>VAL</u>	G + +2	<u>LEU</u> ser <u>GLN</u> pro <u>LYS</u> ile <u>VAL</u> lys <u>TRP</u>
F + 0	HIS thr <u>VAL</u> gln <u>CYS</u> ser <u>TYR</u>	F + 0	HIS asn <u>VAL</u> arg <u>CYS</u> ala <u>TYR</u>
C + -2	<u>VAL</u> thr <u>VAL</u> ala <u>TRP</u> lys	C + -2	asp <u>ILE</u> glu <u>VAL</u> asp <u>LEU</u>
FALC OBSERVED		FALV OBSERVED	
A +	ser <u>VAL</u> thr <u>LEU</u> phe <u>PRO</u>	B +	GLY thr <u>CYS</u> ser <u>ILE</u> thr <u>VAL</u> arg
B +	ASP ser <u>ILE</u> leu <u>CYS</u> val <u>LEU</u> thr <u>ALA</u>	F +	SER ser <u>ALA</u> thr <u>LEU</u> ala <u>ILE</u>
E +	lys <u>TYR</u> ala <u>ALA</u> ser <u>SER</u> tyr <u>LEU</u> ser <u>LEU</u> thr	E +	ser <u>LYS</u> ser <u>VAL</u> ser <u>PHE</u>
D +	gln <u>LYS</u> ser <u>PRO</u> thr <u>THR</u> glu <u>VAL</u> gly	A +	PRO ser <u>VAL</u> ser <u>GLY</u>
G +	SER thr <u>VAL</u> glu <u>LYS</u> thr <u>VAL</u>	H +	<u>LEU</u> arg <u>VAL</u> phe <u>GLY</u> gly <u>THR</u> lys <u>LEU</u> thr <u>VAL</u> leu
F +	HIS thr <u>VAL</u> gln <u>CYS</u> ser <u>TYR</u>	G +	ASP tyr <u>SER</u> gln <u>CYS</u> tyr <u>TYR</u> asp <u>ALA</u>
C +	<u>VAL</u> thr <u>VAL</u> ala <u>TRP</u> lys	C +	lys <u>TRP</u> tyr <u>GLN</u> gln
		D +	ile <u>LEU</u> leu <u>LYS</u>
FAHC OBSERVED		FAHV OBSERVED	
A +	lys <u>GLY</u> ser <u>VAL</u> phe <u>PRO</u> leu	A +	<u>LEU</u> glu <u>GLN</u> ser
B +	phe <u>TYR</u> lys <u>VAL</u> leu <u>CYS</u> gly <u>LEU</u>	B +	ser <u>VAL</u> thr <u>CYS</u> thr <u>LEU</u> ser <u>LEU</u>
E +	leu <u>TYR</u> ser <u>LEU</u> ser <u>SER</u> val <u>VAL</u> thr	G +	<u>PHE</u> ser <u>LEU</u> arg <u>LEU</u>
D +	gln <u>LEU</u> val <u>ALA</u> phe <u>THR</u> his <u>VAL</u> gly	F +	leu <u>MET</u> thr <u>VAL</u>
G +	<u>THR</u> lys <u>VAL</u> asp <u>LYS</u> lys <u>VAL</u>	I +	<u>CYS</u> ile <u>ASP</u> trp <u>GLY</u> gln <u>SER</u> leu <u>VAL</u> thr
F +	<u>HIS</u> asn <u>VAL</u> asn <u>CYS</u> ile <u>TYR</u>	H +	<u>LEU</u> asn <u>ARG</u> ala <u>CYS</u> tyr <u>TYR</u> val <u>ALA</u>
C +	<u>VAL</u> thr <u>VAL</u> ser <u>TRP</u> asn	C +	ASP tyr <u>TYR</u> thr <u>TRP</u> val <u>ARG</u> gln
		D +	phe <u>VAL</u> tyr <u>GLY</u> <u>ILE</u> <u>TRP</u> glu <u>LEU</u>
		E +	thr <u>SER</u> asp <u>THR</u> asp

Fig. 2 Strand-alignment diagrams for β -sheets. The relative positions of the strand residues are shown. β -bulges²⁸ are indicated as in Pro Thr in strand D of FALC. Residues with side chains pointing towards the other sheet are in capital letters. In the diagrams, observed area changes on sheet sandwiching are indicated by enclosing the residue in a rectangle if the area change is $>10\text{\AA}^2$ and by underlining if the area change is between 5 and $10\text{\AA}^2$. In the predicted diagrams, the central residues are in rectangles whilst the other residues that form the site are underlined. The relative phasings of the central residues are shown. For the light (B2-M) and heavy (AC-2) chains, our proposed models are described. The secondary structure prediction³¹ located the β -regions in B2-M as: 1-12, 22-30, 34-39, 48-50, 61-65, 69-71, 80-89 and residues 41-47 as α -helix. The predicted β -regions in the AC-2 fragment were: 5-9, 16-24, 30-35, 46-51, 58-66, 76-81, 88-89. The potential Φ -area of a site is the sum of the available areas for the 2, 3 or 4 component nonpolar residues. The area for a nonpolar residue X is the available Φ -area of X in a model, three-stranded twisted antiparallel sheet $(\text{Cys}_3)_2:(\text{Cys}_4\text{XCys}_4):(\text{Cys})_3$. This area agrees well with the observed change for X on sheet/sheet association. The values ($\text{\AA}^2$) we calculated are: Ala(8), Cys(11), Ile(18), Leu(19), Met(26), Phe(24), Pro(9), Trp(34), Tyr(17), Val(13) and Lys(12) if it occurs in an edge strand. Other amino acids were designated as polar and assigned zero area.

then the magnitude of their possible hydrophobic contribution to sheet/sheet stacking from the available Φ -area (see legend to Fig. 2).

(2) *Construction of all possible strand-alignment diagrams.* Next strands are placed in the two β -sheets. As not all the strands contribute markedly to the sheet/sheet interaction (Fig. 2), the sandwich initially was modelled as a central hydrophobic core of two 3-stranded β -sheets. First, all possible topologies for the two sheets are generated. Each β -strand with a hydrophobic site can occupy any position and direction

in either β -sheet. Next, all alignments of the β -strands are generated so as to produce the correct directionality of the anti-complementary parallelograms observed on sheet sandwiching. This was quantified by 32 allowed phasings (see legend to Table 1). For example, in FALC the relative positions of central residues in strands ABE, GFC are 0, 0, +2 and 0, 0, -2 (see Fig. 2). Between 10^6 and 10^7 different strand-alignment diagrams were considered since the information from steps 1 and 2 has reduced the number of possibilities by two orders of magnitude (Table 1). From each

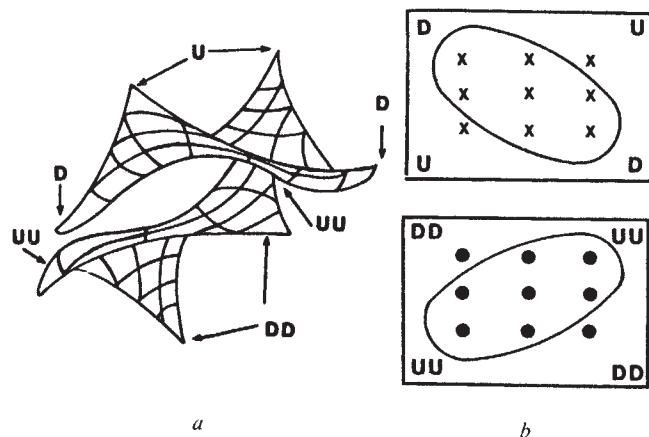


Fig. 3 The stacking of β -pleated sheets. *a*, Two twisted β -sheets with the up (U, UU) and the down (D, DD) corners of the top (one letter) and bottom (two letters) sheet. The lines on the surfaces convey curvature. *b*, The crosses and dots indicate the sheet residues that point to the other sheet. The β -strands run horizontally. The packing of the down corners of the top sheet against the up corners of the bottom sheet produces the observed anti-complementary 'parallelograms' of area changes. Note these area changes cannot be produced by simply superimposing the two ovals in the planar representation as the sheets will then pack with a positive angle rather than the observed negative angle.

central core a set of complete structures was constructed with the previously unplaced strands being located in every possible unoccupied position and with every direction. No attempt was made to phase the unplaced strands with respect to the central core. Clearly, a different approach is required for the 14 strands in CONA.

(3) *Construction of a C^α representation.* C^α coordinates can be obtained from a predicted strand-alignment diagram for the central core. Each β -sheet was built from idealized twisted β -strands²⁶ based on aligning the positions of the central residues. The two sheets were sandwiched by aligning the central residues of the middle strands and placing the sheets at a typical separation (10 Å) and rotation (-30°) suggested by analysis of the structures.

(4) *Constraints on allowed structures.* The list of generated structures is filtered by imposing the following restrictions that quantify observed topological (i, ii, iii), steric (iv, v) and hydrogen-bonding (vi, vii) features of known β -sheet sandwiches. (i) The connection between two parallel β -strands in the same sheet is right-handed^{3,4}. (ii) Connections between β -strands do not cross²⁷. (iii) The topology of the strands includes a generalized 'Greek-key'^{6,27}. (iv) There are sufficient residues between the strands to make the required connection length. (v) Any disulphide bridge must be between residues whose C α atoms are closer than 15 Å (a conservative estimate to allow for errors in the C α construction). In addition, a survey of known protein structures suggests that the disulphide bridge is not allowed between cystine residues in non-adjacent β -strands in the same β -sheet (J. M. Thornton, personal communication). (vi) Given the strand composition of the sheets but not the strand positions, structures must make either all or all but one of hydrogen bonds that could be

formed. (vii) The two β -sheets in the central core must have a high degree of strand overlap. This notion was quantified as the number of main-chain hydrogen bonds formed as a fraction of the number of main-chain nitrogen (or oxygen) atoms in the sheet. Structures allowed by filters (i) to (vi) were rank ordered on strand overlap. We determined the position of the native structure in the list.

Results of the prediction of sheet sandwiches

The computer program was uniformly applied to the nine sandwiches to yield reduced lists of allowed sheet sandwiches rank ordered on strand overlap. For each sandwich, one member of the reduced list has all or most of the relative residue positions in the native strand-alignment diagram. The deviations are generally caused by β -bulges²⁸ that disrupt the register of some residues in a β -strand with respect to the central core. Apart from β -bulges, seven of the nine sandwiches are correctly built. The exceptions are in FCH2 where one strand is shifted by two residues, and in FAHV where one strand is one residue away from the correct alignment.

The strand-alignment diagrams contain sufficient information to construct a C α representation that is close to the native. The r.m.s.d. between the equivalenced C α atoms ranges from 1.4 to 4.9 Å, with 34 to 51 residues being placed. A random prediction¹⁵ of a compact structure for pancreatic trypsin inhibitor (54 residues) would have a mean and standard deviation of the r.m.s.d. of 11.9 + 1.5 Å.

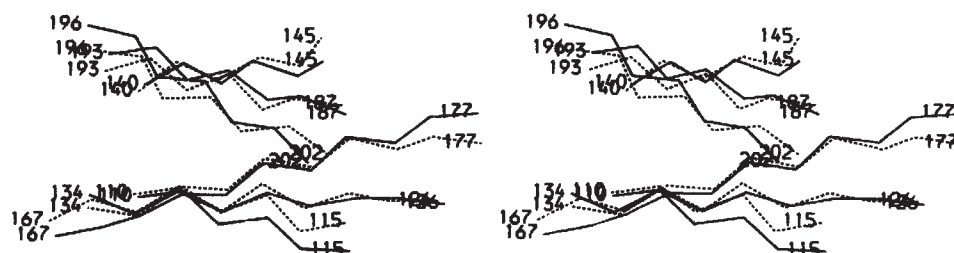
Table 1 indicates the selectivity of the filters by giving the number of central core structures that have no less than the strand overlap of a selected approximation (see above) to the actual structure. For four of the nine sandwiches the algorithm is highly selective and <50 alternative structures (<20 different topologies) would need to be surveyed to locate the central core of the native structure. The algorithm is fairly selective (<3,500 structures, <500 topologies) for the other sandwiches.

Application to the prediction of unknown structures: the histocompatibility antigens

The notions quantified in the algorithm provide, for the first time, the basis for taking a secondary structure prediction and obtaining a tertiary fold for β -sheet sandwiches. Clearly further work on the overall scheme is required. (1) Secondary structure prediction algorithms must be improved to obtain a better location of the β -strands. (2) Given the predicted core, the few unplaced β -strands and all the connections have to be located. (3) The use of simplified energy calculations¹¹⁻¹³ must be explored first to select the correct fold from the reduced list and subsequently to refine the structure.

We will demonstrate the status of this approach by assessing the validity of predicted strand-alignment diagrams for the light chain β_2 -microglobulin (B2-M) and a segment of the heavy chain (AC-2) of the HLA-B7 antigen^{29,30}. Based on sequence alignments, it has been proposed that both B2-M and AC-2 will have a 4- on 3-stranded sheet sandwich structure similar to the immunoglobulin constant domains.

Fig. 4 Predicted and crystal structures of FALC. Stereo diagram of the C α positions of the two three-stranded core of the predicted sandwich (solid lines) superimposed on the crystal structure (broken lines) for FALC.



First, we predicted the secondary structure by the procedure of Robson and coworkers³¹. With suitable decision constants (more than 50% β -structure and less than 20% α -helix), trials of IgG domains localized most β -strand regions. The predicted β -strands in B2-M (see legend to Fig. 2) correspond to β -strands in IgG constant domains when the sequences are aligned as in ref. 29. From this alignment, with only slight modifications to the secondary structure, we constructed the strand-alignment diagram in Fig. 2. The predicted central residues of the hydrophobic sites have the correct anti-complementary direction. Furthermore, probable β -bulges are located in strands A and B in equivalent positions to observed bulges in FAHC and FCH3. When this approach was followed with the AC-2 sequence, we could not construct a plausible strand-alignment diagram. Trials with a sequence alignment program similar to that used for the AC-2/IgG comparisons failed to obtain the correct alignment of residues between FAHC and FAHV²¹. Accordingly, we modified the proposed sequence alignment of AC-2/IgG to obtain a strand-alignment diagram that had suitable features of hydrophobic patches and β -bulges (Fig. 2). The diagrams were taken as the 'native' structure of B2-M and AC-2, and the algorithm for predicting sheet sandwiches then established that these structures are one of a small list (<100) of allowed sandwiches compatible with the β -strand assignment (Table 1). Predicted C α coordinates of the strands in B2-M and AC-2 are available upon request.

Packing of α -helices against pure parallel β -sheets

The remaining structural motif frequently observed in globular proteins involves the packing of α -helices against a predominantly parallel β -sheet. Calculations of Φ -area change are in progress. In flavodoxin, the independent docking of each α -helix against the native 5-stranded sheet yields Φ -area changes equivalent to between -10 and -20 kcal mol⁻¹. This hydrophobic effect lies between that of sheet sandwiching (-24 kcal mol⁻¹ for prealbumin) and α -helix/ α -helix packing (-13 kcal mol⁻¹ for an interaction in myoglobin⁷). The α -helix/ β -sheet interaction involves Φ -area changes of residues in at least three adjacent strands. This is because the van der Waals diameter of an α -helix is 10 Å whilst adjacent β -strands lie about 4.25 Å apart.

There are also features that reduce the number of allowed topologies. For structures with a repeating unit $(\beta x)_n$ where x is either an α -helix or an irregular coil region and where all the β -strands lie parallel, there are two known restrictions. (1) The handedness of the connection between parallel β -strands is nearly always right-handed^{3,4}. (2) There is never more than one chain reversal in strand order⁶. Thus ABEDC is acceptable but ABDEC is not (letters A to E denote the sequential order of the β -strands). A survey of nine parallel β -sheets (Table 2) found two additional rules: (3) The difference in the number of α -helices on the two surfaces of the β -sheet is never more than 1. (4) The position of an α -helix trailing the last β -strand in the β -sheet can be fixed. To remove rotational symmetry the second strand is placed to the right of the first. The trailing α -helix will lie on the top of the β -sheet and to the left of the first strand; otherwise all the α -helices could not pack against the β -sheet.

In structures with n parallel strands and $(n-1)$ connections there generally are $2^{(n-1)}n!/2$ topologies^{3,4,32} for the strands and connections (Table 2). In addition, the option to form a β -barrel for sheets of more than six β -strands doubles the number of topologies. The four rules drastically limit the number of allowed topologies to less than 25. Barrels with eight strands, for example triose phosphate/isomerase, must show the topology ABCDEFGH with no chain reversals (compare ref. 6). We are developing an algorithm to predict hydrophobic docking sites of α -helices and β -sheets. Thus we plan to extend the combinatorial approach to α/β structures.

Implications for protein evolution and structure prediction

We have demonstrated that there are severe restrictions on the number of allowed topologies both for sheet sandwiches and for α -helices packing against a pure parallel β -sheet (Tables 1 and 2). Therefore the similarity of structure between Cu:Zn superoxide dismutase and the immunoglobulins³³ and the related topologies of some glycolytic enzymes (see for example refs 3, 4, 32, 34) could adequately be explained as the selection of similar structures from the small list of allowed folds. Thus, in our opinion, to favour the alternative explanation of divergence from a common ancestor requires different proteins that are either β/β or α/β structures to have a very similar topology and highly significant sequence resemblances.

The combinatorial approach to structure prediction is radically different from relying solely on energy minimization. To fold a protein correctly starting from an extended structure, with possibly preset α -helices and β -strands, requires an algorithm whose radius of convergence is at least the diameter of the compact globule. Such convergence is unlikely to be achieved by energy minimization due to the problem of being trapped in a local minimum. In the combinatorial approach, all structures that obey semi-empirical rules for the tertiary fold are sampled and thereby one obtains a list of possible models that should always include the rough fold of the native. For at least the proteins that belong to one of the well-defined structural classes, further work on the lines reported might well provide a solution to the protein folding problem.

We thank Sir David Phillips for encouragement and advice, and Professor F. M. Richards and Dr T. J. Richmond for useful discussion and the solvent accessibility program. The MRC provided financial support and the SRC and Oxford University provided computing facilities. F.E.C. is a Rhodes Scholar, M.J.E.S. a Stothert Fellow of the Royal Society and W.R.T. has a post-graduate studentship from Northern Ireland.

Received 28 December 1979; accepted 1 April 1980.

- Levitt, M. & Chothia, C. *Nature* **261**, 552-558 (1976).
- Chothia, C. & Janin, J. *J. molec. Biol.* **105**, 1-14 (1976).
- Richardson, J. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2619-2623 (1976).
- Sternberg, M. J. E. & Thornton, J. M. *J. molec. Biol.* **105**, 367-382 (1976); **110**, 269-283 (1977).
- Chothia, C., Levitt, M. & Richardson, D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4130-4134 (1977).
- Richardson, J. S. *Nature* **268**, 495-500 (1977).
- Richmond, T. J. & Richards, F. M. *J. molec. Biol.* **119**, 537-555 (1978).
- Némethy, G. & Scheraga, H. A. *Q. Rev. Biophys.* **10**, 239-352 (1977).
- Sternberg, M. J. E. & Thornton, J. M. *Nature* **271**, 15-20 (1978).
- Schulz, G. E. & Schirmer, R. H. *Principles of Protein Structure* (Springer, New York, 1979).
- Levitt, M. *J. molec. Biol.* **104**, 59-107 (1976).
- Kuntz, I. D., Crippen, G. M., Kollman, P. A. & Kimelman, D. *J. molec. Biol.* **106**, 983-994 (1976).
- Robson, B. & Osguthorpe, D. J. *J. molec. Biol.* **132**, 19-51 (1979).
- Hagler, A. T. & Honig, B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 554-558 (1978).
- Cohen, F. E. & Sternberg, M. J. E. *J. molec. Biol.* **138**, 321-333 (1980).
- Cohen, F. E., Richmond, T. J. & Richards, F. M. *J. molec. Biol.* **132**, 275-288 (1979).
- Cohen, F. E. & Sternberg, M. J. E. *J. molec. Biol.* **137**, 9-22 (1980).
- Nyberg, S. C. *Acta crystallogr.* **B30**, 251-253 (1974).
- Bernstein, F. C. *et al.* *J. molec. Biol.* **112**, 535-542 (1977).
- Saul, F. A., Amzel, L. M. & Poljak, R. J. *J. biol. Chem.* **253**, 585-597 (1978).
- Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M. & Palm, W. *Nature* **264**, 415-420 (1976).
- Blake, C. C. F., Geisow, M. J., Oatley, S. J., Rerat, B. & Rerat, C. *J. molec. Biol.* **121**, 339-356 (1978).
- Beale, D. & Feinstein, A. *Q. Rev. Biophys.* **9**, 135-180 (1976).
- Chothia, C. *J. molec. Biol.* **75**, 295-302 (1973).
- Momany, F. A., McGuire, R. F., Burgess, A. W. & Scheraga, H. A. *J. phys. Chem.* **79**, 2361-2381 (1975).
- Pitts, O. B., Finkelstein, A. V. & Falk Bendzko, P. *FEBS Lett.* **101**, 1-5 (1979).
- Richardson, J. S., Getzoff, E. D. & Richardson, D. C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2574-2578 (1978).
- Dayhoff, M. O. *Atlas of Protein Sequence and Structure* Vol. 5 Suppl. 2, 168-169 (National Biomedical Research Foundation, Washington D.C., 1976).
- Orr, H. T., Lancet, D., Robb, R. J., Lopez de Castro, J. A. & Strominger, J. L. *Nature* **282**, 266-270 (1979).
- Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97-120 (1978).
- Schulz, G. E. & Schirmer, R. H. *Nature* **250**, 142-144 (1974).
- Richardson, J. S., Richardson, D. C., Thomas, K. A., Silvertown, E. W. & Davies, D. R. *J. molec. Biol.* **102**, 221-235 (1976).
- Rossmann, M. G., Liljas, A., Branden, C.-I. & Banaszak, L. J. *Enzymes* **11**, 61-102 (1974).

LETTERS

Periodicity of the γ -ray transient event of 5 March 1979J. Terrell, W. D. Evans, R. W. Klebesadel
& J. G. Laros

University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

An unusual γ -ray burst event was observed on 5 March 1979 by nine different spacecraft^{1,5}. The position of the event has been accurately determined^{1,2} as $\alpha = 5^{\text{h}} 25.95^{\text{m}}$, $\delta = -66^{\circ}07.1'$ (epoch 1950.0), coincident with the location of the supernova remnant N49 in the Large Magellanic Cloud. The burst was of very high intensity, and if isotropic and located at the distance (~ 55 kpc) of N49 had a peak luminosity of $> 10^{44} \text{ erg s}^{-1}$. Even more interesting is the obvious 8-s periodicity^{3,5} of the event, following the initial very intense outburst. We report here the time history and power spectrum of this event as determined from the Pioneer Venus Orbiter (PVO) data.

PVO had the best available time resolution during the initial peak and a long usable record of clearly detectable oscillations. The PVO γ -ray burst detector⁶ is sensitive to γ rays in the energy range 60–1,200 keV and has a rate-dependent resolving time which can be as low as 0.244 ms. The counts obtained during a γ burst are stored in the spacecraft memory for later transmission.

Figure 1a shows part of the PVO record of the 5 March event, as counts grouped into uniform 11.72-ms intervals (the largest resolving time in memory mode) and corrected for an effective dead time of 6 μs . The γ -ray flux rose to near its maximum in a time ≤ 1 ms (possibly much less), a fact which is not apparent in Fig. 1 because of the grouping of data. The even smaller rise time of < 0.25 ms which was originally reported^{1,3} corresponds to the minimum resolving time, which was not achieved until the peak of the outburst. The γ -ray flux remained near the maximum level for about 30 ms, fell to a somewhat lower level for about 70 ms, and then decayed with an ~ 35 -ms decay time to a level about 200 times lower. The γ rays had energies extending above 300 keV during the initial outburst, and a spectral distribution which can be fitted reasonably well by an exponential in photon number with $kT \approx 60$ keV (E. E. Fenimore, personal communication). The maximum counting rate, which has an uncertainty of $\sim 10\%$ due to dead-time corrections, corresponds to $\sim 3 \times 10^{-3} \text{ erg cm}^{-2} \text{ s}^{-1}$. The data obtained at high time resolution in memory mode, for 3 s before and 21 s after the outburst, are only partially displayed in Fig. 1.

Following this outburst, which lasted about 200 ms, the γ -ray flux oscillated with an 8-s period for at least 180 s (ref. 3), at a level initially about 500 times less than at the peak, and with a softer spectrum⁷. Figure 1b shows the counts grouped in 2-s intervals (as transmitted in 'real time' mode) for 200 s, beginning 19 s before the γ burst. There are, clearly, high peaks in the counting rate every 8 s, beginning 4 s after the initial

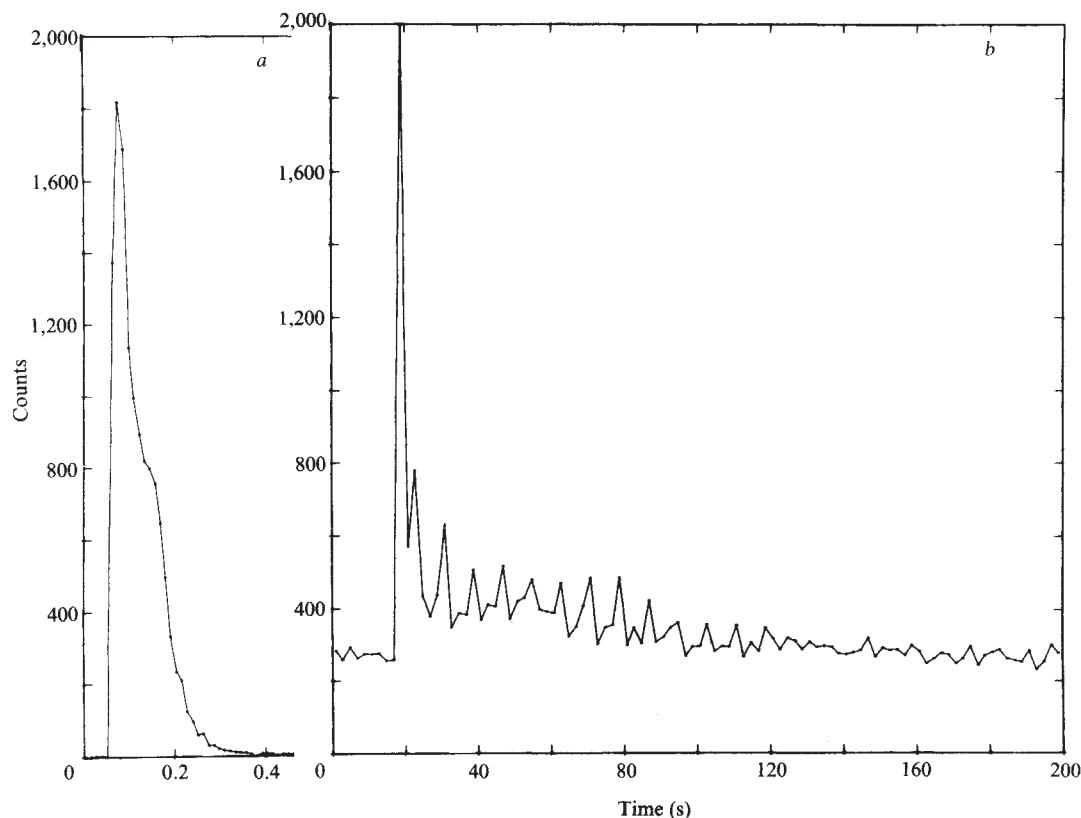


Fig. 1 Initial time history of the 5 March 1979 event (a) as seen by PVO. The counts have been grouped in uniform 11.72-ms time intervals for Fourier analysis, so that the ≤ 1 ms rise time is not apparent here. b, PVO real-time history of the same event, with counts grouped into 2-s bins. For clarity, the actual peak count of 13,000 is not fully shown. The energy range covered is 60–1,200 keV.

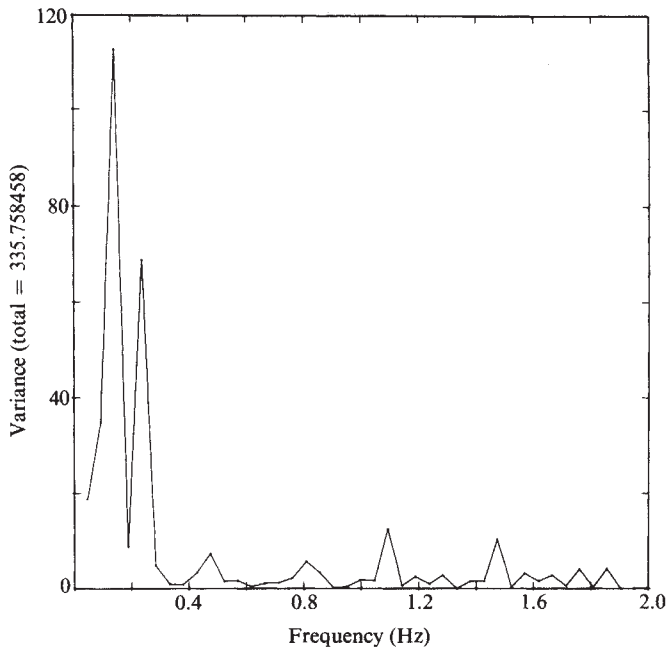


Fig. 2 Power spectrum of the 5 March 1979 event, from 21 s of high-time-resolution PVO data immediately after the peak outburst. The data have been grouped into 0.25-s bins and detrended for this analysis, but no smoothing has been used.

outburst, with lower interpulses every 8 s which evidently begin with the shoulder following the initial peak in Fig. 1.

Because of the clear evidence of periodicity, the counting data, both for memory and real-time modes, have been subjected to extensive Fourier analysis. The 21 s of high-resolution data obtained from PVO after the initial outburst yield a power spectrum, seen in Fig. 2, with clear evidence of periods of 4 and 8 s and no other periods significantly above the counting noise level. These peaks are more sharply defined in the longer time history available in real time (2-s resolution) counting mode, as seen in Fig. 3, which shows the power spectrum obtained from 120 s of data immediately following the initial outburst. The spectrum is presented as variance at each multiple of the fundamental frequency (0.00833 Hz) corresponding to the 120-s time series. No smoothing or fading have been used in these analyses; the methods used are more fully discussed elsewhere^{8,9}. The data have been subjected to linear detrending before analysis to remove the very low frequency peak which would otherwise appear in the power spectrum, some trace of which can still be seen at the lowest frequencies.

The two very clearly displayed peaks in Fig. 3, at frequencies of 0.125 and 0.250 Hz, correspond to the fundamental period and its second harmonic. The peaks in this spectrum are so narrow that their width is seen only in the analyses which we have done of longer sets of the PVO data. The broad base visible around each peak is very similar to what is obtained in the analysis of decaying sine waves, performed as tests of the methods used here. Thus the shape of the peaks is essentially lorentzian, although (Fig. 1b) the decay of the oscillations is clearly not precisely exponential, nor is it the same for main pulse and interpulse; the interpulse even seems to be growing during the first few periods. The second harmonic is stronger in the long time series, undoubtedly because of the relatively higher interpulses. The second harmonic in Fig. 3 falls exactly at the Nyquist frequency of 0.250 Hz (for 2-s time resolution), above which there is no additional spectral information. However, as seen in Fig. 2, analysis of PVO data with higher time resolution shows no

significant evidence of higher frequencies. The counting noise level in Fig. 3 corresponds to an average variance of 12 (per Fourier harmonic), which is negligible compared with the peaks.

We have done numerous other power-spectrum analyses of the data of this γ -burst event, and of simulated sets of data, for varying lengths of time series and for various sub-intervals of the data. When the time series does not cover an exact multiple of the 8.0 s period, even when differing by 1 time unit out of 120, the power spectrum peaks are noticeably broadened and lowered, as would be expected for a sharp periodicity. Many such analyses of our data have made it clear that the best estimate of the period is 8.00 ± 0.05 s, in agreement with the result of Cline *et al.*³ This also agrees well, of course, with the 7.9 ± 0.3 and 8.1 ± 0.1 s periods reported by Barat *et al.*⁴ and by Mazets *et al.*⁵, but is more precisely known because of the long time series available from the PVO and ISEE 3 spacecraft.

The power spectrum of this event is thus quite consistent with a decaying periodicity with a well defined period of 8.0 s. The strong second harmonic at 4.0 s is to be expected from the pulse shape with its obvious interpulse. There is no evidence of other harmonics in any of the data. Such a power spectrum is more likely to be produced by a rotating source than by some type of resonance. For a rotating astronomical source, centrifugal force at the surface must be less than gravitational force, leading to the equation (for a spherical source of period T)

$$\rho \geq 3\pi/GT^2 \quad (1)$$

in which ρ is the source density and G is the gravitational constant. A rotating source with $T=8$ s must thus have $\rho \geq 2.2 \times 10^6$, which could be consistent with a white dwarf as source. A more stringent limit is set by the ≤ 1 -ms rise time of this outburst, which suggests that the radius of the source is probably $< 1,000$ km. For a source of roughly one solar mass this requires a density $\rho \geq 10^9$, which is consistent only with a

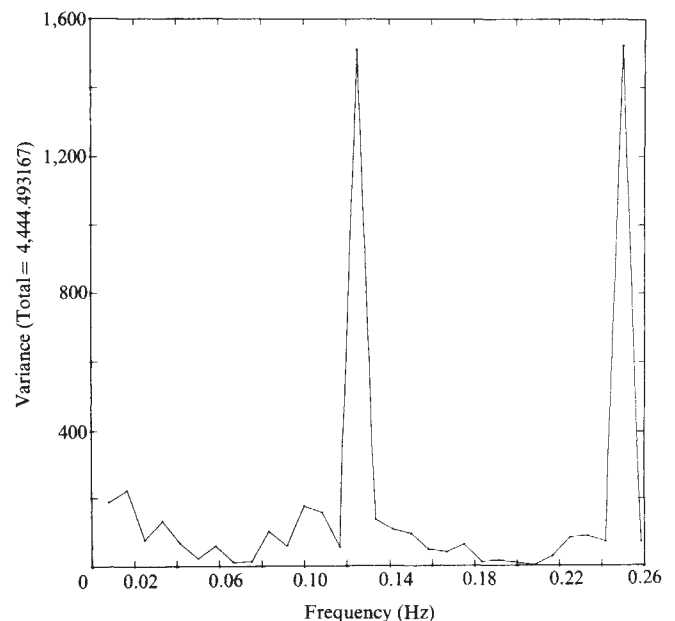


Fig. 3 Power spectrum of the 5 March 1979 event, based on 120 s of PVO data immediately following the initial outburst, with counts in 2-s groups. The data were detrended before analysis. The spectrum, presented as variance (for each Fourier harmonic), has not been smoothed.

neutron star, or with a gravitationally collapsed object of even higher density.

Thus it seems likely that the γ -ray transient event of 5 March 1979 originated in a neutron star of rotation period 8.0s, perhaps an old pulsar. The direction of this source is coincident with the supernova remnant N49, although the distance of the source remains in some doubt^{2-5,10}. It seems reasonably certain that there were two oppositely placed source regions on the rotating neutron star, perhaps at the magnetic poles. The occurrence of three additional outbursts from the same source^{5,11} on 6 March, 4 April and 24 April 1979 indicates that the cause of the outbursts is related to characteristics of the neutron star itself (such as an internal restructuring⁷, or a critical accumulation of accreted material) and not due to improbable recurring collisions with incoming objects.

We thank E. R. Tech and S. E. Duran for help with data processing, J. P. Glore, F. J. Wymer and R. E. Spalding for electronic design and E. E. Fenimore and R. Ramaty for useful discussions. This work was supported by the US Department of Energy and by NASA.

Note added in proof: The difficulty of radiating sufficient flux from the surface of a neutron star at the distance of the LMC^{5,10,12} is avoided if the surface is at a temperature of ~ 30 keV, since the radiation pressure within the surface would be sufficient to blow off the surface layer to a depth of many optical mean free paths. The relativistically-expanding shell could readily emit the required flux, becoming optically thin before it reaches the maximum radius (40,000 km) determined¹³ from the effective pulse length of 0.13 s.

Received 11 February; accepted 14 March 1980

1. Evans, D. *et al.* *IAU Circ.* No. 3356 (1979).
2. Evans, W. D. *et al.* *Astrophys. J. Lett.* **237**, L7-L9 (1980).
3. Cline, T. L. *et al.* *Astrophys. J. Lett.* **237**, L1-L5 (1980).
4. Barat, C. *et al.* *Astr. Astrophys.* **79**, 124-125 (1979).
5. Mazets, E. P., Golenetskii, S. V., Il'inskii, V. N., Aptekar', R. L. & Guryan, Yu. A. *Nature* **282**, 587-589 (1979).
6. Evans, W. D. *et al.* *Science* **205**, 119-121 (1979).
7. Fenimore, E. E., Evans, W. D., Klebesadel, R. W., Laros, J. G. & Terrell, J. *Nature* (submitted).
8. Terrell, J. & Olsen, K. H. *Astrophys. J.* **161**, 399-413 (1970).
9. Terrell, N. J. *Astrophys. J. Lett.* **174**, L35-L41 (1972).
10. Helfand, D. J. & Long, K. S. *Nature* **282**, 589-591 (1979).
11. Golenetskii, S. V., Mazets, E. P., Il'inskii, V. N. & Guryan, Yu. A. *Astr. Zh.* (submitted).
12. Petschek, A. & Colvin, J. D. Preprint.
13. Terrell, J. *Astrophys. J.* **213**, L93-L97 (1977).

The IR spectrum of the double QSO

M. J. Lebofsky*, G. H. Rieke†, D. Walsh‡
& R. J. Weymann*

*Steward Observatory, and †Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721

‡University of Manchester, Nuffield Radio Astronomy Laboratory, Jodrell Bank, Macclesfield, Cheshire, UK

The hypothesis that the double QSO, 0957+561A,B (separation 6 arcs and $z=1.4$) is formed by a gravitational lens¹ has found strong support from observations² suggesting the presence of an intervening giant elliptical galaxy with $z \approx 0.4$, separated from component B by < 1 arcs. According to the lens hypothesis the flux ratio of the two QSOs should be the same at all wavelengths. Any difference observed should be due only to superposition on component B of emission from the lens galaxy, which is expected to be predominantly in the IR. We report here new photometry at 1.25, 1.6 and 2.2 μ m which strengthens the case for lens action and confirms that the lens galaxy is a giant elliptical.

IR photometry of the double QSO was obtained on 28 November 1979 with the 2.29-m telescope at Steward Observatory. The photometer utilized a liquid helium cooled InSb detector, 5.8 arcs diameter aperture, and interference

Table 1 IR photometry of the double QSO

	1.25 μ m	1.60 μ m	2.2 μ m
Component A (north)	$0.61 \pm 0.04^*$	0.80 ± 0.03	0.61 ± 0.04
Component B (south)	0.71 ± 0.04	1.11 ± 0.04	1.19 ± 0.03

*All fluxes in mJy = 10^{-29} W m⁻² Hz⁻¹, errors are 1 σ deviation.

filters defining the standard photometric bands *J* (1.25 μ m), *H* (1.6 μ m), and *K* (2.2 μ m). The measurements were relative to reference areas 9 arcs to the east and west of the double QSO components. The seeing was ~ 1 arcs and the night was of excellent photometric quality. The flux was reduced by standard star observations^{3,4}. The measurements are summarized in Table 1.

In the radio⁵⁻⁷ and blue and UV^{2,8} the B (southern) component of the QSO is 75% as bright as the A component. Beginning abruptly at 5,500 Å, the B component becomes progressively redder towards longer wavelengths; for example, at 7,000 Å with 7 arcs aperture the observed ratio B/A is close to unity².

Our new IR observations with 5.8 arcs aperture show a striking increase in the flux ratio B/A to ~ 2 at 2.2 μ m. We can test whether this is consistent with lens action by a giant elliptical galaxy as follows. We assume a constant intrinsic flux ratio B/A of 0.75. Using the published optical² and our IR data we subtract 0.75 of the flux of A from that of B. The excess flux from B is then expected to be the contribution from the galaxy. The derived galaxy flux is compared with the spectral energy distribution typical of nearby giant ellipticals^{9,10} in Fig. 1. The standard elliptical energy distribution was redshifted by $z=0.4$, with appropriate *K* corrections¹¹. The galaxy near the double QSO can be seen to have an energy distribution in the *J*, *H*, *K* bands that is slightly redder than the typical nearby elliptical, and to have a distinct excess in the visible relative to the IR flux.

To facilitate further comparison with other galaxies at $z=0.4$, the *R* colour in the Johnson system was computed from the optical data², and a small aperture correction was applied from our 5.8 arcs to the 7 arcs aperture used in the optical. The colours of this galaxy are given in Table 2. For comparison, a sample of four giant elliptical galaxies with z ranging from 0.38 to 0.42 (A370, 0822+67, 1613+31, and 0949.9+4409) have both IR and optical colours measured¹¹⁻¹³. The average colours for this group of ellipticals are also given in Table 2, where the errors are indicative of the

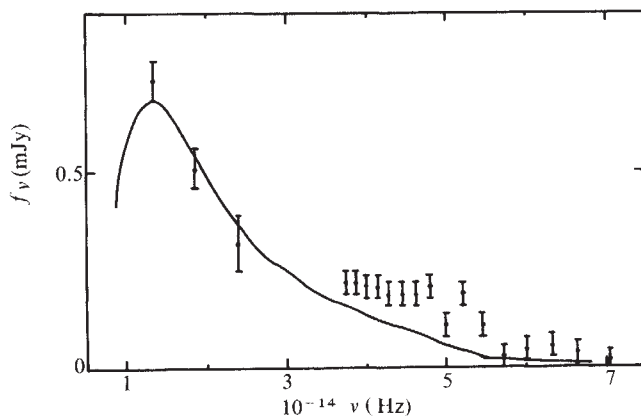


Fig. 1 The spectrum of the excess of component B of the double QSO above 75% of the flux of component A. Data points are shown by dots, with 1- σ error bars. Errors for the data at frequencies above 3×10^{14} Hz (ref. 2) have been estimated by comparing the spectra of components A and B. The solid line shows the spectrum of a typical nearby giant elliptical galaxy, redshifted to $z=0.4$ and normalized to fit the IR data.

Table 2 Colours of redshifted giant elliptical galaxies

	R-K	J-H	H-K
Nearby galaxy shifted to $z=0.4$	3.55	1.07	0.75
Galaxies observed at $z=0.4$	3.3		0.93 ± 0.06
Galaxy associated with component B	3.1	1.3 ± 0.3	0.9 ± 0.1

spread in colours and uncertainty in aperture correction between optical and IR data. Note that there are differences of up to 0.25 mag between the colours of giant ellipticals actually observed at $z=0.4$ and those estimated from the known properties of nearby giant ellipticals. It is not appropriate to discuss this in detail here, but the effect seems to be real. However, the measured colours of galaxies at $z=0.4$ are a very close match to the observed colours of the galaxy near component B of the double QSO. The absolute magnitude of the galaxy at K can be estimated from the observed magnitude of $K=14.9$. Because the guiding during the observation of the southern component was done visually on the QSO, the galaxy was not centred in the beam, making an exact aperture correction difficult. A value of 0.5 mag has been adopted¹⁴, which yields $M_K \sim -26.1$ for $q_0=0$, $H_0=100$. The values of M_K for giant ellipticals range from $M_K=-25.7$ to $M_K=-26.8$ (refs 9, 14) making this galaxy within the giant elliptical luminosity range.

The photometry of component A of the double QSO in Table 1 shows nearly equal fluxes at J and K with a larger flux at H. $H\alpha$ should be shifted to $1.58\mu\text{m}$ at $z=1.41$. If $I(\text{Crv})/I(H\alpha)=0.5$, a typical value for QSOs¹⁵, then the excess at H above a spectrum interpolated from J to K corresponds exactly to the expected contribution from $H\alpha$. The spectrum of the QSO from optical to IR wavelengths is remarkably flat when compared with other QSOs. For example, a power law fitted to the spectrum of component A from 0.44 to $2.2\mu\text{m}$ has an index of $\alpha=-0.15$, compared with the normal range of $-0.5 \geq \alpha \geq -1.5$ (ref. 16).

A crucial test of the gravitational lens hypothesis is that the two images of the QSO should have identical spectra. At present, in the IR it is technologically feasible to test this prediction only over the 1–2.5 μm spectral region. Our measurements demonstrate that the IR spectra are consistent with the QSO images having a constant flux ratio except for contamination by a giant elliptical galaxy² of luminosity and colour typical for similar galaxies at the same redshift. The IR measurements therefore support the gravitational lens model of the double QSO.

This work was supported by the NSF. GHR is an Alfred P. Sloan Fellow.

Anti-matter in the primary cosmic radiation

J. Szabelski & J. Wdowczyk

Institute of Nuclear Research, Uniwersytecka 5, Lodz, Poland

A. W. Wolfendale

Department of Physics, University of Durham, Durham DH1 3LE, UK

Anti-matter in the cosmic radiation incident on the Earth can be of two types: antiparticles from distant antigalaxies and secondary particles generated by collisions of cosmic rays with nuclei of the interstellar medium (ISM) either in our Galaxy or in other galaxies. Insofar as the 2.7 K relic radiation inhibits the former for positrons (with energy above some tens of MeV) and the expected ratio of extragalactic particles to galactic particles is very small for protons (and thus antiprotons) it is conventional to assume that collisions in the ISM are the source of the e^+ and $\bar{p}$ detected. We consider here the manner in which cosmic rays are propagated in the Galaxy by studying the fluxes of these antiparticles.

The 'leaky box' model is the simplest one for cosmic ray propagation; in it there is essentially equilibrium between the particles and their secondaries, both being lost by leakage after many collisions with the 'boundary' of the Galaxy. In this model both the primary cosmic rays and their secondaries have the same mean lifetime in the Galaxy and the amount of material traversed (the 'grammage' mean density of gas $\times$ mean lifetime $\times$ velocity) is the same for them all.

We have previously examined the positron results and concluded that there is evidence against the 'leaky box' model of propagation¹. We showed specifically that the average

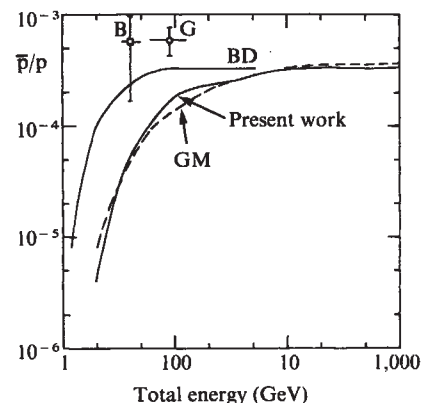


Fig. 1 Comparison of observed and expected ratios of antiprotons to protons in the primary cosmic ray beam. G denotes the measurement of Golden *et al.*² and B that of Bogomolov *et al.*³. The former is 15% higher than our value because of our use of a correction for atmospheric $\bar{p}$ derived from the present calculations rather than those of Badhwar *et al.*⁵. The latter is 5% lower than our value because they ignored an atmospheric correction. The predictions relate to the ratios expected for 5 gm^{-2} of interstellar hydrogen traversed by the cosmic rays using the leaky box model (that is the intensity of $\bar{p}$ is that generated by the locally measured cosmic ray spectrum—corrected for solar modulation—in 5 gm^{-2} of ISM). BD denotes Badhwar *et al.*⁵ (for a primary spectrum of exponent $\gamma=2.75$), GM denotes Gaisser and Maurer⁴ ($\gamma=2.6$). In the present work the adopted primary spectrum has a variable exponent. We find it very significant that the observed ratio is much higher than our predicted ratio.

Received 31 January; accepted 28 March 1980.

- Walsh, D., Carswell, R. F. & Weymann, R. J. *Nature* **279**, 381–384 (1979).
- Young, P., Gunn, J. E., Kristian, J., Oke, J. B. & Westphal, J. A. *Astrophys. J.* (submitted).
- Johnson, H. L. *Commun. Lunar Planet. Lab. Univ. Arizona* No. 53 (1965).
- Low, F. J. & Rieke, G. H. in *Methods of Experimental Physics* Vol. 12 (ed. Carleton, N.) 415–462 (Academic, New York, 1974).
- Pooley, G. G. *et al. Nature* **280**, 461–464 (1979).
- Roberts, D. H., Greenfield, P. F. & Burke, B. F. *Science* **205**, 894 (1979).
- Porcas, R. W., Booth, R. S., Browne, I. W. A., Walsh, D. & Wilkinson, P. N. *Nature* **282**, 385–386 (1979).
- Wills, B. J. & Wills, D. *Astrophys. J.* (in the press).
- Frogel, J. A., Persson, S. E., Aaronson, M. & Matthews, K. *Astrophys. J.* **220**, 75–97 (1978).
- O'Connell, R. W. Preprint (1980).
- Lehovsky, M. J. in preparation.
- Gunn, J. E. & Oke, J. B. *Astrophys. J.* **195**, 255–268 (1975).
- Kristian, J., Sandage, A. & Westphal, J. A. *Astrophys. J.* **221**, 383–394 (1978).
- Persson, S. E., Frogel, J. A. & Aaronson, M. Preprint (1980).
- Baldwin, J. A. *Astrophys. J.* **214**, 679–684 (1977).
- Neugebauer, G., Oke, J. B., Becklin, E. E. & Matthews, K. *Astrophys. J.* **230**, 79–94 (1979).

grammage traversed by positrons was significantly shorter than that traversed by nuclei, of the same rigidity, when both were calculated adopting the leaky box model.

The present analysis was prompted by the recent determination of the flux of antiprotons in the cosmic radiation by Golden *et al.*², and by Bogomolov *et al.*³. The initial objective is to take the best estimate of the primary spectra of the various cosmic ray components ($p, \alpha, \dots$) together with nuclear physical data on $\bar{p}$ production in the relevant particle collisions and to derive the expected $\bar{p}$ spectrum for the traversal of 1 g cm^{-2} of ISM (or 1 g cm^{-2} of hydrogen-equivalent). This is compared with the observed $\bar{p}$ spectrum, and the mean grammage required ($\langle \lambda \rangle_{\bar{p}}$) is determined. (In the leaky box model this is simply the observed $\bar{p}$ intensity divided by the predicted $\bar{p}$ intensity for 1 g cm^{-2} traversed.) Two previous calculations have been made using modern $\bar{p}$ data^{4,5} but with such discordant results, that we analysed the input data rather critically.

The cross-sections were taken from the Durham-Rutherford data base and comprised all the available data on inclusive reactions $pp \rightarrow \bar{p} + \text{anything}$ and $p - \text{light nucleus} \rightarrow \bar{p} + \text{anything}$, the latter being converted to expectation for pp . The energy range for initiating proton extended from 19.2 GeV (CERN experiments⁶) by way of 70 GeV (Serpukhov⁷) and 100–400 GeV (Fermilab⁸) to 2,096 GeV (ISR⁹) (these refs are only a selection of the many data sources used). Integration was carried out over transverse momentum and $\bar{p}$ multiplicity times inelastic cross-section was derived for various proton energies and $\bar{p}$ energy thresholds, as given in Table 1. The data are accurate to $\sim 15\%$ over the important energy range—which is partly confirmed by the estimation by Rossi *et al.*¹⁰, who give the yield of $\bar{p}$ of all energies as a function of proton energy (Table 1). The cross-section ($\sigma_{in} \times \langle n \rangle(\bar{p})$) is usually slightly more than the flux we calculated for $E_{\bar{p}} > 1.5 \text{ GeV}$. (Note that the basic data are almost the same as those used by Badhwar *et al.*⁵, the main addition is new results from Fermilab.)

The cosmic ray proton spectrum adopted is similar to that used in our earlier work, viz: $J(E) = 2 \times 10^4 E^{-2.75} (1 + 4.26 E^{-0.876})^{-1} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ GeV}^{-1}$ where E is the kinetic energy.

For α particles a ratio of intensities of $\alpha/p = 0.05$ was adopted above 20 GeV per nucleon, following the summary by Juliusson¹¹ (this differs slightly from our earlier assumption but not significantly so). The spectra of heavier cosmic ray nuclei have also been taken from Juliusson.

The expected energy spectrum of antiprotons from $p-p$ collisions follows from using the data of Table 1 with $J(E)$. Assuming equal production of $\bar{n}$ and $\bar{p}$ the detected flux of $\bar{p}$ will be higher than calculated by a factor 2 (the $\bar{n}$ decay into $\bar{p}$) and when allowance is made for α particles and heavier nuclei a further factor 1.25 must be applied to give the expected flux of $\bar{p}$ per g cm^{-2} of H (or H-equivalent) in the ISM. (Note, the factor 1.58 adopted by Badhwar *et al.* relates to flux per g cm^{-2} of ISM in total and should not be compared with our factor 1.25; it does not matter which factor is used as long as the same factor is used for the grammages for the other components with which comparison is made.)

Figure 1 shows the derived $\bar{p}/p$ ratio from the present work for the traversal of 5 g cm^{-2} H-equivalent together with the other predictions^{4,5} for the same grammage, and the observations^{2,3}.

Although we have considered the predictions of the other workers referring to the same target material, hydrogen (the ratio of Badhwar *et al.* has been multiplied by 1.25/1.58 and that of Gaisser and Maurer by 1.25), they refer to different primary spectra, and the comparison must be made with care.

Gaisser and Maurer used a primary spectrum with constant exponent ($\gamma = 2.6$) and this is not too far from our primary spectrum over much of the energy range. Their predicted $\bar{p}/p$ ratio is seen to be not far from ours and this indicates that our derived cross-sections for $\bar{p}$ production are similar. An

accurate comparison has been made, in fact, by using the data of Table 1 with a primary spectrum having $\gamma = 2.6$; for antiprotons of energy in the range 5–10 GeV (the important range in practice) the ratio of the GM value of $\bar{p}/p$ to ours is 0.61. The same ratio for $\bar{p}$ of all energies is 0.74. The difference in the results is greater than we would have expected from uncertainties in the cross-sections ($\sim 20\%$), but not dramatically so.

The difference in Fig. 1 between the prediction of Badhwar *et al.* and ours is bigger than can be explained in terms of the differing primary spectrum (they used $\gamma = 2.75$). Reworking our prediction for $\gamma = 2.75$ gives the ratio of their value of $\bar{p}/p$ (for 5–10 GeV) to ours of 2.7 and the ratio for all energies is 6.1. This discrepancy is hard to understand as we seem to have used the same sources of accelerator data for cross-sections for $\bar{p}$ production. Their roughly constant $\bar{p}/p$ ratio down to a $\bar{p}$ energy as low as $\sim 5 \text{ GeV}$ is particularly hard to understand; it would correspond to a plot of total number of $\bar{p}$ versus proton energy at variance with accelerator data at proton energies below about 100 GeV.

Assuming that our calculations are correct, it is easy to derive the values of $\langle \lambda \rangle_{\bar{p}}$ required by the two experimental measurements of $\bar{p}/p$ (Fig. 1) (they are simply the measured $\bar{p}/p$ ratios divided by the predictions, multiplied by 5 g cm^{-2}) assuming that the leaky box model is valid.

Comparison can now be made with the mean grammages derived from the mass composition analyses ($\langle \lambda \rangle_N$) and positron data ($\langle \lambda \rangle_{e+}$) in the same way. Although there is some spread of values of $\langle \lambda \rangle_N$ from different workers, most lie in the range shown (see refs 12–14 for representative results). Concerning $\langle \lambda \rangle_{e+}$, we have used our analysis of the expected positron flux and its comparison with observation. The value of $\langle \lambda \rangle_{e+}$ is sensitive to the mean lifetime of electrons in the Galaxy and we have adopted a value of $\langle T \rangle = 2 \times 10^7 \text{ yr}$ for this quantity¹⁵.

It is apparent that there is no consistency between the various values of $\langle \lambda \rangle$ such as would have been expected. Admittedly, there are uncertainties in the analysis but, what is probably the biggest—the spectrum in interstellar space of cosmic ray protons—cancels out when $\langle \lambda \rangle_{\bar{p}}$ and $\langle \lambda \rangle_{e+}$ are compared. The most likely explanation is a lack of validity of the leaky box model^{1,16}. An alternative explanation is that the bulk of the antiprotons are of extragalactic origin (our evidence on the shape of the $\bar{p}$ spectrum does not preclude this) but it seems rather unlikely both because of the above arguments, and because the inequality of $\langle \lambda \rangle_{e+}$ and $\langle \lambda \rangle_N$ indicates propagation effects, and such effects can also make $\langle \lambda \rangle_{\bar{p}} \neq \langle \lambda \rangle_N$. There may also be problems with excessive γ -ray fluxes if the postulated extragalactic flux of $\bar{p}$ has high flux at low energies.

Table 1 Cross-section (mb per proton) for $\bar{p}$ produced with energy above $E_{\bar{p}}$, for proton–proton collisions, versus incident proton energy, E_p

E_p (GeV)	15	20	30	50	100	200	500	1,000
$E_{\bar{p}}$ (GeV)								
1.5	$< 10^{-2}$	0.083	0.18	0.35	0.80	1.75	3.8	6.4
5	$< 10^{-3}$	0.014	0.073	0.18	0.57	1.40	3.4	5.9
10	$< 10^{-3}$	0.0072	0.054	0.32	0.92	2.8	5.2	
σ_{in} (mb)	29.7	30.0	30.3	30.8	31.4	32.0	33.1	34.5
$\langle n \rangle(\bar{p})$	2×10^{-3}	2.8×10^{-3}	5.6×10^{-3}	1.2×10^{-2}	2.8×10^{-2}	6.0×10^{-2}	1.2×10^{-1}	1.5×10^{-1}

The cross-section is $\langle n \rangle \sigma_{in}$ where $\langle n \rangle$ is the mean multiplicity of $\bar{p}$ above $E_{\bar{p}}$ and σ_{in} is the inelastic cross-section for $p-p$ collisions, σ_{in} is also given, as is the average number of $\bar{p}$ of all energies, $\langle n \rangle(\bar{p})$. (The latter is from ref. 10.)

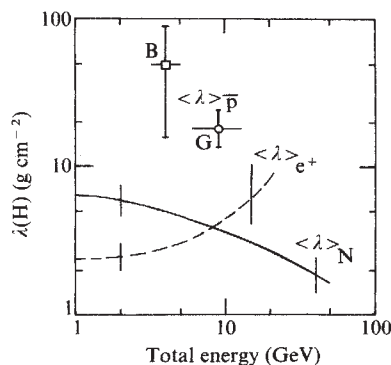


Fig. 2 Mean grammage versus total energy for cosmic rays incident on interstellar hydrogen, derived using the leaky box model of cosmic ray propagation. $\langle \lambda \rangle_p$ comes from the present analysis, $\langle \lambda \rangle_{e^+}$ is taken from our earlier work¹. The vertical bars indicate the order of accuracy of $\langle \lambda \rangle_N$ and $\langle \lambda \rangle_{e^+}$. The lack of agreement strongly supports the idea that the usually adopted leakbox model is not valid and that cosmic ray propagation is more complex.

There are several ways of explaining Fig. 2 in terms of propagation effects. An attractive model is one in which protons (and probably α particles) have sources which differ in general from those of heavier nuclei. These sources could be differently distributed in space and/or time; for example, the old idea of production of protons in intermittent Galactic centre 'explosions' is attractive. This would result in protons and heavier nuclei having different distributions in the Galaxy, and the grammage 'seen' by those particles detected at the Earth would be different. A long 'tail' to the lifetime distribution would not be surprising and this would depress the positron flux and thus the $\langle \lambda \rangle_{e^+}$ values shown in Fig. 2 because of the removal of positrons by synchrotron and inverse Compton losses; such losses would be quite natural if the particles spent a considerable time in the galactic halo.

Another possibility relating to propagation is the inclusion of deceleration of particles after they have accumulated the bulk of their grammage; because of the different spectral shapes of p and $\bar{p}$, and $\bar{p}/p$ ratio at low energies ($E < 10$ GeV) would be significantly enhanced by deceleration. What is quite clear is that the opposite effect is ruled out², viz that the bulk of the grammage is encountered before acceleration; the $\bar{p}/p$ ratio at low energies would be very small under such circumstances. Although the deceleration idea does not explain the e^+ results (for which we feel that a long lifetime 'tail' is the most likely explanation) it does have attractive features and needs further analysis.

The antiproton measurements give further evidence against the simplistic leaky box model of cosmic ray propagation and point to a more complex manner of propagation, probably involving considerable fractions of particle lifetimes spent in the galactic halo. An alternative explanation in terms of extragalactic antiprotons from antigalaxies although unlikely cannot be ruled out. A more extended measurement of the energy spectrum of $\bar{p}$ (and e^+) would probably enable a distinction to be made.

Received 28 February; accepted 14 April 1980.

- Giler, M., Wdowczyk, J. & Wolfendale, A. W. *J. Phys.* **A10**, 843 (1977).
- Golden, R. L. *et al. Phys. Rev. Lett.* **43**, 1196 (1979).
- Bogomolov, E. A. *et al. Proc. int. Cosmic Ray Conf.*, Kyoto 1, 330 (1979).
- Gaisser, T. K. & Maurer, R. H. *Phys. Rev. Lett.* **30**, 1264 (1973).
- Badhwar, G. D. *et al. Astrophys. Space Sci.* **37**, 283 (1975).
- Allaby, J. V. *et al. Proc. 4th Int. Conf. on H.E. Collisions*, Oxford 2, 85 (1972).
- Antipov, Yu. M. *et al. Phys. Lett.* **34B**, 2, 164 (1971).
- Johnson, J. R. *et al. Phys. Rev.* **D17**, 5, 1292 (1978).
- Capiluppi, P. *et al. Nucl. Phys.* **70**, 1 (1974).
- Rossi, A. M. *et al. Nucl. Phys.* **B84**, 269 (1975).
- Juliusson, E. *Proc. int. Cosmic Ray Conf.*, Munich 8, 2689 (1975).
- Shapiro, M. M. & Silberberg, R. *Proc. R. Soc.* **277**, 319 (1974).
- Caldwell, J. H. *Astrophys. J.* **218**, 269 (1977).
- Garcia-Munoz, M. *et al. Proc. int. Cosmic Ray Conf.*, Kyoto 1, 310 (1978).
- Garcia-Munoz, M., Mason, G. M. & Simpson, J. A. *Astrophys. J. Lett.* **201**, L141 (1975).
- Giler, M., Wdowczyk, J. & Wolfendale, A. W. *J. Phys.* **A11**, 199 (1978).

Frequency of the methane-stabilized He-Ne laser at 88 THz measured to ± 3 parts in 10^{11}

D. J. E. Knight*, G. J. Edwards, P. R. Pearce & N. R. Cross

National Physical Laboratory, Teddington, Middlesex TW11 0LW, UK

We have measured the frequency of a methane-stabilized laser at 88 THz (3.39 μ m) (ref. 1), by a method which largely eliminates the uncertainties contributed by lower-frequency lasers used as transfer oscillators. This result is primarily of interest for a proposed new definition of the metre in which the speed of light in vacuum (c_0) would be kept constant and reference would be made to the standard of time and frequency². For this reason our measurement has been referred to the mean frequency of five small portable, stabilized lasers of similar design that are used as wavelength standards^{3,4}. We estimate the mean frequency of the laser ensemble to have been $(88,376,181,616 \pm 3)$ kHz. The estimated standard deviation comes within about a factor of 2 of the reproducibility of the lasers themselves. This result therefore demonstrates a degree of accuracy that would permit the choice of a definition of the metre in which c_0 was kept constant, without substantial loss of accuracy compared with choosing a definition based on the wavelength of a selected stabilized laser.

The methane-stabilized helium-neon laser¹ at 3.39 μ m is one of a growing group of stabilized lasers recommended for use as wavelength standards². Although it lacks a visible radiation, it has a high degree of reproducibility^{1,3,4}, approaching 1 part in 10^{11} , and its frequency has been directly measured by comparison with the caesium standard using harmonic generation and frequency-mixing techniques^{5,6}. Frequency and wavelength measurements on both this and a carbon dioxide stabilized laser⁷ have established the presently accepted value of the speed of light². The uncertainty in c_0 , ± 4 parts in 10^9 , arises from the realization of the metre from the ⁸⁶Kr lamp², and is 10 times larger than was obtained for the frequency of the methane-stabilized laser. Since then wavelength-ratio determinations against other stabilized lasers have become more accurate than the methane frequency measurements^{8,9}.

The measurement described here was obtained using a simplified chain of comparison having only four main transfer oscillators (one a klystron) between the methane-stabilized laser and a rubidium standard. Further details appear elsewhere^{10,11}.

The frequencies are related by the four mixing equations

$$f_{\text{CH}_3\text{OH}} = 43f_{99} \pm 120 \text{ MHz} \quad (1)$$

$$f_{R32} = 7f_{\text{CH}_3\text{OH}} - 3f_{95} \pm \Delta f_b \quad (2)$$

$$f_{\text{He-Ne}} = 3f_{R32} - f_{55} \pm \Delta f'_b \quad (3)$$

and

$$f_{\text{CH}_4} = f_{\text{He-Ne}} \pm \Delta f''_b \quad (4)$$

where $f_{\text{CH}_3\text{OH}}$, f_{R32} , $f_{\text{He-Ne}}$ and f_{CH_4} are laser frequencies at 4.25, 29.5, 88.4 and 88.4 THz, f_{99} , f_{95} and f_{55} are klystron frequencies suffixed in GHz, and Δf_b , $\Delta f'_b$ and $\Delta f''_b$ are measured beat frequencies in the 10–100-MHz band. The mixing devices (one per equation) were (1) a Josephson junction, (2) and (3) metal-insulator-metal diodes and (4) a photodiode.

*Present address: Bureau International des Poids et Mesures, F-92310 Sèvres, France.

The beat signals were obtained sufficiently strongly to be counted with digital counters, and this in turn allowed us to make our measurements simultaneously (with a 1 s averaging time) and so eliminate the effects of frequency changes among the three transfer lasers, which were used free-running. Simultaneous counts were made on Δf_b , $\Delta f'_b$, $\Delta f''_b$ and on f_{99} , that of a klystron phase-locked to the lowest-frequency laser. The four experimenters closely monitored the beats and the state of phase lock of the klystron during the counts. f_{95} and f_{55} were controlled by quartz crystals and were measured 1 s later. A computer (Hewlett Packard 9825A) was linked to the counters and performed the arithmetic. The rubidium standard that controlled the counters was calibrated at intervals of about 2 days by carrying it to a caesium clock in another building, and the methane-stabilized laser (NPL1) was checked against another soon after the measurements and against others during the preceding 12 months.

Measurements were taken on three nights over a period of 5 days. The results of the third night are split into two groups because of an alteration made in the counting system to permit greater resolution of a critical quartz-crystal frequency controlling f_{95} . A summary of the data for the four groups, corrected for the rubidium offset and a counter offset (see below), is shown in Table 1.

Within each group the measurements were made in blocks of 10, with a quasi-random permutation between each block of the various ($\pm$) sidebands possible in the frequency chain. For each group, tests of the homogeneity of the variances and means (Bartlett's test, F -test) of the subsidiary blocks are entirely satisfactory at the 5% level. However, an F -test on the means of the four groups leads to an F -value corresponding to a probability of 5%, which indicates some inconsistency between them. Group 3b differs from the others in having a smaller standard deviation, which was the result of the change in the apparatus mentioned above, and in having an offset mean, which caused us to look for possible systematic errors in the apparatus. We found only a small error in one of the counters, shown as a 0.7-kHz correction in Table 2, but there was also evidence of a trend with time in the results, beginning in set 3a and continuing through set 3b. Parameters such as the frequency of the methane-stabilized laser or of the rubidium clock might be expected to change more between groups than within them, so, following Eisenhart¹², we take the unweighted mean of the four means of the groups. We obtain $f - f_0 = +8.8$ kHz with an estimated standard deviation of 2.0 kHz. However, as there are only four groups this value has a large uncertainty, of the order of 40%. The standard deviation corresponds to a scatter between the groups of about 2 kHz, which is rather larger than the contributions expected from the rubidium clock and stabilized laser, about 1 kHz each. However, it was not possible during these measurements to check the stabilized laser against a second

Table 1 Summary of the measurements

Group	Day (August 1979)	Time (UT)	n^*	Mean [†] ($f - f_0$) (kHz)	σ_{single} (kHz)	σ_{mean} (kHz)
1	17/18	22.30–04.00	41	+12.9	32.3	5.0
2	20/21	22.30–02.30	124	+9.4	34.5	3.1
3a	22/23	21.00–02.00	110	+9.4	29.2	2.8
3b	23	03.00–04.30	49	+3.4	11.7 [‡]	1.8

*About 30% of all attempted measurements were rejected at the time of measurement because of faults seen while monitoring the apparatus. A few results (about 4% in groups 1, 2 and 3a; 11% in Group 3b) were further rejected during analysis by applying a $\pm 3\sigma_{\text{single}}$ cutoff to the data.

[†] $f_0 = 88\,376\,181\,608$ kHz (an arbitrary reference zero). The means have been corrected for the rubidium clock offset and the error in a counter (see Table 2).

[‡]The counting resolution was increased for this group, see text.

Table 2 Summary of corrections* and uncertainties (kHz)

Stage of comparison	Mean correction	Uncertainty (estimated standard deviation)		
		Statistical (n)	Estimated	Total
Caesium versus rubidium	+8.5	0.7 (5)	1.1	1.3
Rubidium versus laser	+0.7 [†]	2.0 (4) [‡]	0.1	2.0
Laser versus ensemble	−1.0	0.7 (5)	1.0	1.2
Total	+8.2	2.2	1.5	2.7

*The first two corrections listed have already been incorporated into Table 1. The final result is obtained by applying the third correction to the overall mean of Table 1, see text.

[†]Applies to groups 1, 2 and 3a only.

[‡]Derived from the four group means in Table 1, see text.

similar one, and further, it counts against group 3b that it spans a short period of time compared with the others.

The corrections applied to the data and the main contributions to the overall uncertainty are listed in Table 2. The uncertainty deduced from Table 1 appears in the central line; the first two corrections are already incorporated in the mean values given in Table 1. The main correction (+8.5 kHz, mean) is for the offset of the rubidium clock from its nominal frequency of 10 MHz. The associated statistical uncertainty comes from the scatter of the calibrations and the estimated uncertainty allows for environmental differences between use in our apparatus and being calibrated in the caesium-clock laboratory. The estimated contribution in the second line is associated with the correction and with quartz-crystal drift.

The third correction is applied to give an estimate of the mean frequency of the ensemble of five small lasers mentioned above. Frequency comparisons between these lasers^{3,4}, which include the laser NPL1 used in our measurement, took place over a span of about 12 months, and we restrict the data to those where the lasers were operated using sinusoidal modulation of the cavity length and third-derivative frequency lock. The statistical contribution to the uncertainty reflects the scatter shown in the comparisons, and the estimated one allows for possible offsets in NPL1 when our frequency measurements were made.

Our result for the mean frequency of the ensemble of five lasers is (88, 376, 181, 616 $\pm$ 3) kHz, where the uncertainty is derived as the root sum of squares of the components in Table 2 and approximates one standard deviation of the result.

The uncertainty is so much smaller in this measurement than in the previous ones, by more than a factor 10, that it is capable of exposing previously undetectable sources of systematic error. It agrees well with the earlier results^{5,6}, $+(11 \pm 50)$ kHz and $-(8 \pm 43)$ kHz, respectively, but disagrees with the recent result of Domnin *et al.*¹³, $-(30 \pm 10)$ kHz.

The accuracy of the new methane frequency measurement can in part be transferred to the visible iodine-stabilized lasers at 0.63 μ m through the wavelength ratio, determined by Layer *et al.*⁸. With the latter ratio accurate to ± 2 parts in 10^{10} , we arrive at a situation where the frequencies of the key stabilized lasers used in length measurement are measured within about a factor three or four of their reproducibilities.

We thank B. W. Jolliffe for providing the methane-stabilized lasers, T. G. Blaney for support and some participation in the measurements and D. Sutcliffe, B. Swabey and D. Peacock for help with rubidium clock calibrations.

Received 3 March; accepted 28 March 1980.

1. Barger, R. L. & Hall, J. L. *Phys. Rev. Lett.* **22**, 4–8 (1969).
2. *Com. Consult. Definition Metre* (Com. Int. Poids Mesures) 5th Session (1973); 6th Session (1979).
3. Jolliffe, B. W., Kramer, G. & Chartier, J.-M. *IEEE Trans. Instrum. Meas.* **IM-25**, 447–450 (1976).

4. Felder, R., Jolliffe, B. W. & Chartier, J.-M. *Rapport BIPM/79-7* (Bureau International des Poids et Mesures, F-92310 Sèvres, France, 1979).
5. Evenson, K. M., Wells, J. S., Petersen, F. R., Danielson, B. L. & Day, G. W. *Appl. Phys. Lett.* **22**, 192-195 (1973).
6. Blaney, T. G., Edwards, G. J., Jolliffe, B. W., Knight, D. J. E. & Woods, P. T. *J. Phys. D* **9**, 1323-1330 (1976).
7. Blaney, T. G. *et al. Proc. R. Soc. A355*, 61-68; 89-114 (1977).
8. Layer, H. P., Deslattes, R. D. & Schweitzer, W. G. Jr *Appl. Optics* **15**, 734-743 (1976).
9. Woods, P. T., Shotton, K. C. & Rowley, W. R. C. *Appl. Opt.* **17**, 1048-1054 (1978).
10. Knight, D. J. E. *et al. in Proc. 6th Vavilov Conf. on Nonlinear Optics*, Akademgorodok, Novosibirsk, USSR, June 1979 (in the press).
11. Blaney, T. G., Cross, N. R., Knight, D. J. E., Edwards, G. J. & Pearce, P. R. *J. Phys. D* (in the press).
12. Eisenhart, C. *Natn. Bur. Stand. (U.S.)*, Spec. Publ. 343, 509-518 (1971).
13. Domnin, Yu. S., Koshelyaevskii, N. B., Tatarenkov, V. M. & Shumyatskii, P. S. *Pis'ma v Zh. Eksp. Teor. Fiz. (USSR)* **30**, 273-5 (1979).

Soliton formation and *cis trans* isomerization in polyacetylene

James C. W. Chien, Frank E. Karasz
& Gary E. Wnek

Department of Polymer Science and Engineering,
Materials Research Laboratory, University of Massachusetts, Amherst,
Massachusetts 01003

Polyacetylene can be synthesized enriched in either *cis* or *trans* structure content through varying the temperature of polymerization¹. *Cis* rich polyacetylene is readily isomerized to the *trans* configurations by heating². Such polyacetylene contains a large number of unpaired spins^{3,4} and the paramagnetism has been attributed to a topological soliton⁵, corresponding to a defect in a long-chain polyalkene at which point the Π -wave function amplitude vanishes. Polyacetylene prepared at a low temperature, however, and which has never been exposed to oxygen or subjected to heating and should have a nearly pure *cis* configuration, has been found to lack an EPR signal. We therefore, report here that soliton formation and configurational isomerization are intimately related, and suggest an explanation for both mechanisms.

Acetylene was polymerized as a film at 195 K using the $\text{Ti}(\text{O}-n\text{Bu})_4/\text{AlEt}_3$ catalyst^{1,7}. The freshly prepared film is about 85-87% *cis* as judged from the absorbances of the *cis* and *trans* IR bands at 750 cm^{-1} and $1,010\text{ cm}^{-1}$, respectively ($\% \text{ cis} = 130 A_{\text{cis}} / (1.3 A_{\text{cis}} + A_{\text{trans}})$ ¹). The polymer film was cut into

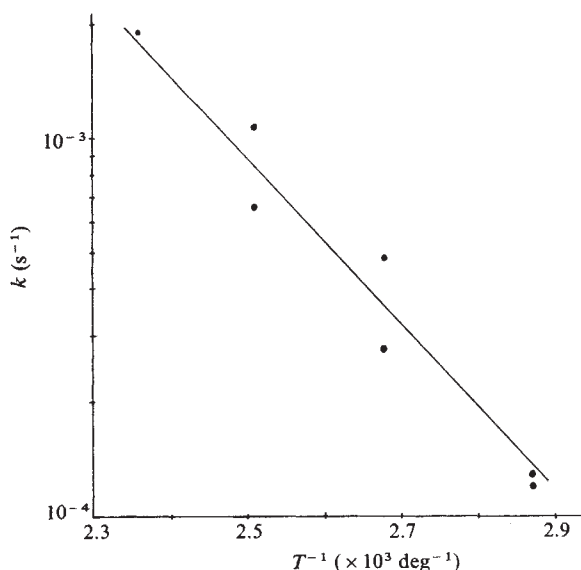


Fig. 1 First order kinetic plot for the formation of unpaired spins in poly(acetylene).

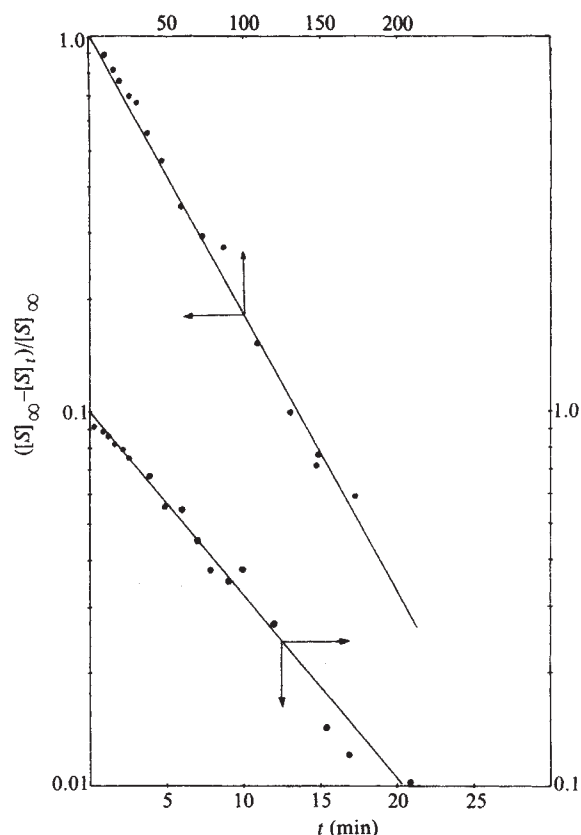


Fig. 2 Arrhenius plot for the rate constant of production of unpaired spins.

strips weighing $\sim 8\text{ mg}$ and transferred to an EPR sample tube which was then capped with a serum stopper and sealed with paraffin wax, all these manipulations being performed in rigorous anaerobic conditions. The EPR signal was monitored with a Varian E-9 X-band spectrometer. By referencing to a specimen of DPPH in the dual cavity one finds a g -value of 2.0022 for the unpaired spin in polyacetylene. Three significant changes are observed on heating: an increase in EPR signal intensity, a decrease in EPR linewidth, and an increase in the *trans* content.

Heating of *cis*-rich polyacetylene resulted in a marked increase in the unpaired spin concentration, $[\text{S}\cdot]$, as obtained by double integration of the EPR signal. Examples at two temperatures (Fig. 1) showed that the formation of the soliton obeys first order kinetics. At any given temperature, $[\text{S}\cdot]$ reaches a certain steady-state value which is unchanged on standing for a reasonably long period at that temperature, and $[\text{S}\cdot]$ does not decrease on cooling the sample. Numerous experiments demonstrated that each incremental temperature of heating results in a new and higher plateau value of $[\text{S}\cdot]$. The apparent first-order rate constants gave a reasonable Arrhenius plot (Fig. 2) and an activation energy of $\sim 10\text{ kcal mol}^{-1}$.

A mechanism of soliton formation may be proposed. *Cis* polyacetylene has a band gap, E_g , of 2.2 eV, suggesting that the molecule has weakly alternating single and double bonds. X-ray diffraction and packing analysis results⁸ are consistent with the following structures:

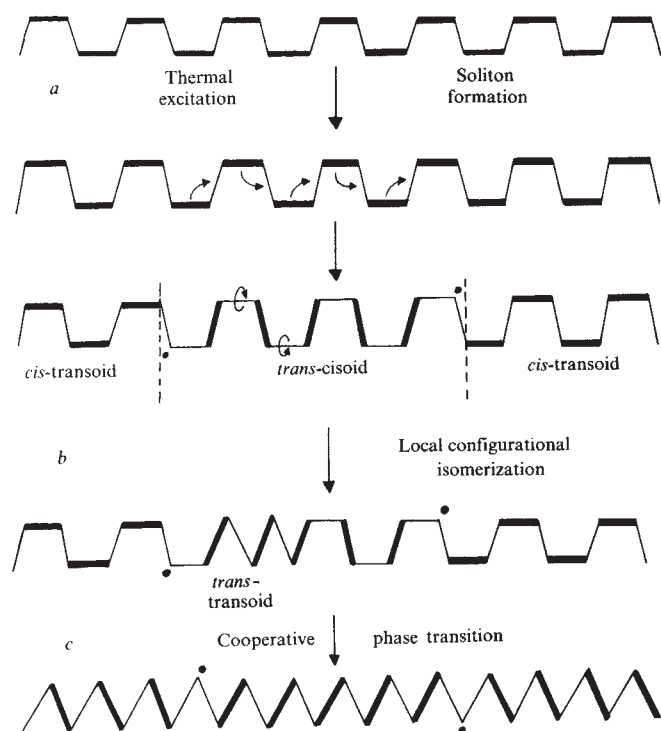


The heavy lines indicate bonds having a length longer than a $\text{C}=\text{C}$ π -double bond, and the lighter lines are shorter than a $\text{C}-\text{C}$ σ -single bond. This partial conjugation lowers E_g .

Table 1 Soliton linewidth

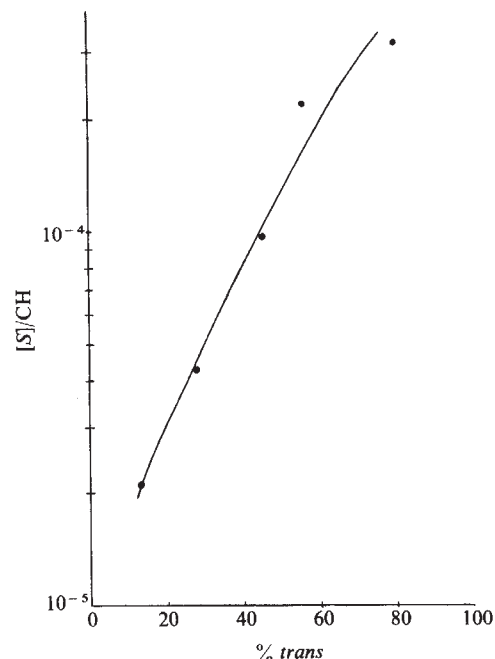
Temperature (°C)	25	50	75	100	125	150
ΔH_{pp} During heating	10	7.4	4.8	3.5	1.3	0.7
ΔH_{pp} After cooled to 25°	10	7.6	5.0	4.5	2.0	1.2

Pristine polyacetylene is relatively free from defects and may be thought of having the idealized structure (I). Thermal excitation produces the soliton by bond rehybridizations as shown in Fig. 3a. The indicated shifting of adjacent long and short bonds results in a sequence of *trans*-*cisoid* segment of structure (II). The formation of such a neutral defect requires a much lower energy, which is estimated to be ~ 0.4 eV (ref. 5), than that required to create a conduction electron or hole, which is $E_g/2$. The lower energy is due to stabilization of the soliton bound hole. As $0.4 \text{ eV} = 9.44 \text{ kcal mol}^{-1}$, the activation energy for the formation of a soliton agrees with the observed activation energy for the increase in $[S\cdot]$.

**Fig. 3** Formation of neutral soliton and isomerization.

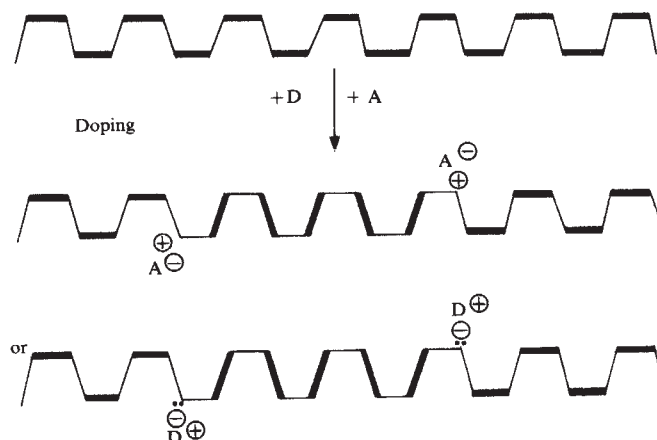
Creation of a soliton is not to be associated directly with configurational isomerization. However, as the defect has a sp^3 free radical-like structure, bond rotation is possible leading to one or more *trans*-*transoid* units (Fig. 3b). Thus, the *trans* content in polyacetylene should increase with $[S\cdot]$ but not in a linear relationship. Figure 4 shows the relationship between $[S\cdot]$ and the *trans* content, the latter determined by IR immediately following the EPR measurements. *Cis*→*trans* isomerization does not obey first order kinetics²; furthermore, the apparent activation energy estimated from the initial rates varied from 17 kcal mol^{-1} for polymers containing 88% *cis* configuration to 39 kcal mol^{-1} for polymers having 80% *trans* content. This may reflect the sequence lengths of *trans*-*transoids* being formed. Unfortunately this sequence length cannot be determined from the most intense out-of-plane CH deformation bands as the frequencies for repeat units (*trans* $\text{CH}=\text{CH}-$)_m(-*cis* $\text{CH}=\text{CH}$)_n with $m, n=2$ are estimated to be within a few per cent of long sequences of *m* and *n* (ref. 9).

Increase of temperature not only generates more solitons but also produces larger domains of *trans*-*cisoid* and/or *trans*-*transoid* units. It follows that the soliton mobility

**Fig. 4** Plot of unpaired spin concentration versus *trans* content.

(delocalization) is enhanced at elevated temperatures. This is supported by the EPR linewidths (ΔH_{pp} peak-to-peak in G, average of three or more experiments); the data are summarized in Table 1. The greater soliton mobility corresponds to narrower linewidth.

In addition to soliton formation and configurational isomerization at intermediate temperatures, there is an irreversible phase transition with a DSC maximum for the exotherm at 145°C . This transition is absent in those *trans* polyacetylenes synthesized at $>150^\circ\text{C}$. During this transition, the *trans*-*cisoids* and short sequences of *trans*-*transoids* are converted to the predominantly *trans* zigzag structure, probably via a cooperative crank shaft type of motion (Fig. 3c). The phase transition is accompanied by changes in the major *d*-spacing in X ray diffraction from $2\theta = 23.15^\circ$ for *cis* polyacetylene to 24.0 – 25.0° for *trans* polyacetylene¹. There is even a 20% increase in length of polyacetylene specimen held under tension with heating which occurs at the temperature range corresponding to the DSC exotherm (H. Skirakawa, personal communication).

**Fig. 5** Generation of charged soliton by doping.

The mechanism proposed above is also pertinent to the conversion of wide band gap semiconducting polyacetylene to highly conducting material by doping¹⁰. Figure 5 shows that the introduction of appropriate dopants (A for acceptor and D for donor) produces charged solitons; at low temperatures, conversion of *cis*-transoid to other configurations can occur as in the previous case of a neutral soliton. A semiconductor-to-metal transition occurs when the charged carrier concentration exceeds the critical value.

We thank Professor A. J. Heeger for helpful discussions, J. D. Capistran for sample preparation, and the Advanced Research Project Agency for support.

Received 7 January; accepted 18 March 1980.

1. Ito, T., Shirakawa, H. & Ikeda, S. *J. Polym. Sci. Polym. Chem. Edn* **12**, 11 (1974).
2. Ito, T., Shirakawa, H. & Ikeda, S. *J. Polym. Sci. Polym. Chem. Edn* **13**, 1943 (1975).
3. Shirakawa, H., Ito, T. & Ikeda, S. *Makromolec. Chem.* **179**, 1565 (1978).
4. Goldberg, I. B., Crowe, H. R., Newman, P. R., Heeger, A. J. & MacDiarmid, A. G. *J. chem. Phys.* **70**, 1132 (1979).
5. Su, W. P., Schrieffer, J. R. & Heeger, A. J. *Phys. Rev. Lett.* **42**, 1698 (1979).
6. Chien, J. C. W., Karasz, F. E., Wnek, G. E., MacDiarmid, A. G. & Heeger, A. J. *J. Polym. Sci. Polym. Lett. Edn* **18**, 45 (1979).
7. Karasz, F. E. *et al. Nature* **282**, 286 (1979).
8. Baughman, R. H., Hsu, S. L., Pez, G. P. & Signorelli, A. J. *J. chem. Phys.* **68**, 5405 (1978).
9. Shirakawa, H. & Ikeda, S. *Polym. J.* **2**, 231 (1971).
10. Chiang, C. K. *et al. J. Am. chem. Soc.* **100**, 1013 (1978).

Table 1 Mass spectra of NPH-ions observed in the stratosphere^{2,4,5} and ion identifications given by Ferguson¹

Observed	Proposed NaOH ion clusters ¹	Proposed KOH ion clusters ¹
42 ± 2 (2) 43 ± 3 (5*)	41 NaOH ₂ ⁺	
60 ± 2 (2, 5*)	59 NaOH ₂ ⁺ · H ₂ O	57 KOH ₂ ⁺
78 ± 1 (4) 78 ± 2 (5*)	77 NaOH ₂ ⁺ · 2H ₂ O	75 KOH ₂ ⁺ · H ₂ O
80 ± 2 (2) 82 ± 2 (5*)	81 NaOH ₂ ⁺ · NaOH	
96 ± 1 (4) 96 ± 2 (5*)	95 NaOH ₂ ⁺ · 3H ₂ O	93 KOH ₂ ⁺ · 2H ₂ O
99 ± 2 (5*) 100 ± 1 (4)	99 NaOH ₂ ⁺ · NaOH · H ₂ O	
114 ± 2 (4)	113 NaOH ₂ ⁺ · 4H ₂ O	111 KOH ₂ ⁺ · 3H ₂ O 113 KOH ₂ ⁺ · KOH
118 ± 1 (4)	117 NaOH ₂ ⁺ · NaOH · 2H ₂ O	129 KOH ₂ ⁺ · 4H ₂ O 131 KOH ₂ ⁺ · KOH · H ₂ O
136 ± 2 (4) 140 ± 2 (4)	135 NaOH ₂ ⁺ · NaOH · 3H ₂ O 139 NaOH ₂ ⁺ · 2NaOH · H ₂ O	

Numbers given in parentheses are references.

*E. Arijis, personal communication to E. Ferguson (see ref. 1).

New gas phase inorganic ion cluster species and their atmospheric implications

T. D. Märk*, K. I. Peterson & A. W. Castleman Jr

Department of Chemistry and Chemical Physics Laboratory, CIRES, University of Colorado, Boulder, Colorado 80309

Recent experimental observations in our laboratory, with high pressure mass spectrometry, have revealed the existence of previously unreported species involving water clustered to sodium dimer ions, and alkali metal hydroxides clustered to alkali metal ions. We discuss here the important implications of these results concerning the existence of such species, and how from a practical aspect they confirm the stability of certain cluster species proposed by Ferguson¹ to explain masses recently detected at upper altitudes using mass spectrometric techniques.

Arnold *et al.*² reported unusual ion masses at altitudes between 37 and 56 km based on rocket-borne mass spectrometric techniques. These ions, NPH (non-proton hydrates), are listed in Table 1. The original suggestion by Arnold *et al.*² that these might be protonated formaldehyde has been discounted³. Later measurements⁴ again led to observations of known proton hydrates (PH) as well as the NPH ions. Additional NPH ions of higher masses were also reported. These ions were proposed to have the general form H⁺X(H₂O)_n and to result from reactions of PH ions with an unknown stratospheric gas X. Acetonitrile (CH₃CN) was tentatively suggested by the authors⁴ as being a possible candidate to explain mass X.

More recently, Ferguson¹ stated that it is difficult to accept acetonitrile as a candidate to explain the NPH ion species and speculated that they might rather be protonated sodium hydroxide cluster ions of the form NaOH₂⁺(H₂O)_n(NaOH)_m. Values of *n* from 0 to 4, and *m* from 0 to 2 allow an interpretation of all seven of the unidentified NPH masses reported by Arnold *et al.*

Arijis *et al.* (ref. 5) have also reported positive ion

measurements based on balloon-borne mass spectrometry (see Table 1). These were made at 35 km elevation after sunset. In addition to observing the normal proton hydrate ions, they reported other ions which correspond in mass to those proposed by Ferguson as being proton hydrates of sodium hydroxides and related higher-order clusters.

Although the proposed identification by Ferguson has been accepted as a likely explanation for the atmospheric observations, as far as we know no evidence of the stability of such higher order clusters involving sodium hydroxide has been given from laboratory experiments. Recent observations in our laboratory confirm not only the existence of alkali metal hydroxides clustered to alkali metal ions but also demonstrate their stability in the presence of water vapour. Proof of the latter fact is crucial in the atmospheric interpretation given by Ferguson. We report some masses which represent other types of inorganic cluster species not previously published.

A high pressure mass spectrometric technique developed⁶⁻⁸ in our laboratory, with a thermionic source, was used to study the three alkali metal ions, sodium, potassium and lithium, clustered with water vapour. These studies were conducted over the temperature range 200–400 °C and at pressures of CO₂ carrier gas corresponding to 5–40 torr; the water concentrations ranged from a few hundredths to ~1%.

Clusters observed in experiments using a sodium ion filament are shown in Table 2, along with proposed identifications. For certain masses greater than 80, an uncertainty arose in assigning their identity because of possible contamination from other species which had been previously present in the apparatus. In particular, some months ago the clustering of dimethoxyethane (DME) to sodium had been studied, and acetone had also been used in certain experiments. Although we believed that these species had been suitably removed by baking and evacuating the system for long periods of time, their presence could not be totally discounted. Therefore, to assist in the identification of these masses and to establish further their assignment as cluster species involving hydroxides, and even the dimers of the alkali metal ions, similar experiments with other alkali metal thermionic sources were made. Especially important were experiments conducted with lithium. Identification of the lower masses of lithium-containing species was unambiguous and

*Permanent address: Institut für Experimentalphysik, Leopold-Franzens-Universität, A-6020 Innsbruck, Austria.

consequently we could make definite assignments with regard to the sodium (and the potassium) system from the results. First, lithium should bond even more strongly with acetone than would sodium. Therefore, failure to detect mass 65 ($\text{Li}^+ \cdot \text{acetone}$) in the lithium experiments enabled mass 81 in the sodium work to be attributed to NaOH_2^+ (NaOH). Second, results with lithium revealed a peak at mass 31 and mass 49. Neither could arise from impurities in the apparatus and confirmed the formation of $\text{Li}^+ \cdot \text{LiOH}$ (31) and $\text{LiOH}_2^+ \cdot \text{LiOH}$ (49).

These results are especially exciting as they confirm the formation and, indeed, the stability of ions involving alkali metals co-clustered with both their corresponding hydroxides, and at the same time water. Another important finding is that the dimer ion of sodium clustered with one water molecule can form in the high pressure reaction source. As seen in Table 2, mass 64 could not be attributed to an impurity and is identified as $\text{Na}_2^+ (\text{H}_2\text{O})$. No higher-order clusters of water about Na_2^+ exist. A related interesting aspect of the results is that no mass 32 ($\text{Li}_2^+ \cdot \text{H}_2\text{O}$) is formed; the first cluster containing two lithium atoms has an attached OH group rather than an H_2O molecule. The reaction of the alkali metal dimer ion with one water molecule is apparently exothermic for the case of lithium to form $\text{Li}^+ \cdot \text{LiOH}$, but requires the addition of a second water molecule in the case of sodium and potassium. This interpretation implies that the bond energy between an alkali metal ion and its corresponding hydroxide is relatively large. Based on thermochemical considerations, the findings indicate that the values exceed $\sim 2\text{eV}$; this is in accord with expectations considering the large dipole moments of the alkali hydroxides. Clearly, these results experimentally confirm the suggested interpretation of the atmospheric measurements¹. Note that solely on a mass basis an alternative interpretation of the NPH ions observed in the stratosphere would be sodium chloride cluster ions of the form $\text{Na}^+ (\text{NaCl})_x (\text{H}_2\text{O})_y$. Values of x from 0 to 2, and y from 0 to 5 allow an assignment for all NPH ions reported. Experiments are in progress in our laboratory to investigate the possibility of the proposed switching reactions and the bond energies for the species identified in this study.

Support of NASA under grant NSG 2248, the Atmospheric Sciences Section of the NSF under grant ATM 79-13801, and the Österreichischer Fonds zur Förderung der Forschung under grant S-18/08 is acknowledged. The Cooperative Institute for Research in Environmental Sciences is jointly sponsored by the University of Colorado and the National Oceanic and Atmospheric Administration.

Table 2 Ion clusters observed in high pressure mass spectrometric studies* of sodium utilizing a thermionic source||

Mass	Proposed identification	Mass	Proposed identification
23	Na^+	113	$\text{Na}^+ (\text{DME})$
41	$\text{Na}^+ (\text{H}_2\text{O})$		$\text{Na}^+ (\text{H}_2\text{O})_5$
59	$\text{Na}^+ (\text{H}_2\text{O})_2$	117	$\text{NaOH}_2^+ (\text{NaOH}) \cdot (\text{H}_2\text{O})_2$
64†	$\text{Na}_2^+ (\text{H}_2\text{O})$	121	$\text{Na}^+ (\text{H}_2\text{O})_3 \cdot (\text{CO}_2)$
67	$\text{Na}^+ (\text{CO}_2)$	131	$\text{Na}^+ (\text{DME}) \cdot (\text{H}_2\text{O})$
77	$\text{Na}^+ (\text{H}_2\text{O})_3$		$\text{Na}^+ (\text{H}_2\text{O})_6$
81‡	$\text{NaOH}_2^+ (\text{NaOH})$	135	$\text{NaOH}_2^+ (\text{NaOH}) \cdot (\text{H}_2\text{O})_3$
85	$\text{Na}^+ (\text{H}_2\text{O}) \cdot (\text{CO}_2)$	139	$\text{Na}^+ (\text{H}_2\text{O})_4 (\text{CO}_2)$
95	$\text{Na}^+ (\text{H}_2\text{O})_4$		$[\text{NaOH}_2^+ (\text{NaOH})_2 \cdot (\text{H}_2\text{O})]_2$
99	$\text{NaOH}_2^+ (\text{NaOH}) \cdot (\text{H}_2\text{O})$	143	$\text{NaOH}_2^+ (\text{NaOH}) \cdot (\text{H}_2\text{O}) \cdot (\text{CO}_2)$
103	$\text{Na}^+ (\text{H}_2\text{O})_2 \cdot (\text{CO}_2)$	149	$\text{Na}^+ (\text{DME}) \cdot (\text{H}_2\text{O})_2$
111	$\text{Na}^+ (\text{CO}_2)_2$		$\text{Na}^+ (\text{H}_2\text{O})_7$

*Experiments were also made with argon and nitrogen as carrier gas, and with deuterated water, to confirm the mass identifications.

†It is unlikely that the observed mass 64 is due to Na^+ attached to an impurity of mass 41 as we would then also expect to see $\text{Na}^+ (41) (\text{H}_2\text{O})$ of mass 82.

‡Corresponds in mass to $\text{Na}^+ \cdot \text{acetone}$. This possibility was discounted on the basis of experiments with lithium where no mass 65 ($\text{Li}^+ \cdot \text{acetone}$) was detected.

§A possible contributor to the more predominant species involving water and carbon dioxide.

|| Present studies.

Received 4 February; accepted 15 April 1980.

1. Ferguson, E. E. *Geophys. Res. Lett.* **5**, 1035 (1978).
2. Arnold, R., Krankowsky, D. & Marlen, K. H. *Nature* **267**, 30 (1977).
3. Fehsenfeld, F. C., Dotan, I., Albritton, D. L., Howard, C. J. & Ferguson, E. E. *J. geophys. Res.* **83**, 1333 (1978).
4. Arnold, R., Böhringer, H. & Henschen, G. *Geophys. Res. Lett.* **5**, 653 (1978).
5. Arijis, E., Ingels, J. & Nevejans, D. *Nature* **271**, 642 (1978).
6. Castleman, A. W. Jr, Holland, P. M., Lindsay, D. M. & Peterson, K. I. *J. Am. chem. Soc.* **100**, 6039 (1978).
7. Castleman, A. W. Jr *Kinetics of Ion-Molecule Reactions* (ed. Ausloos, P.) (Plenum, New York, 1979).
8. Castleman, A. W. Jr *Advances in Colloid and Interface Science* Vol. 10 (ed. Zettlemoyer, A.) 73 (Elsevier, Oxford, 1979).

Heat flow in the Mesozoic and Cenozoic

Donald Sprague & Henry N. Pollack

Department of Geological Sciences, The University of Michigan, Ann Arbor, Michigan 48109

The present-day heat flow from the Earth has commonly been used to estimate the abundance of heat-producing isotopes within the Earth. However, if heat flow has a variability on a short time scale (compared with the isotopic decay times), then the present-day heat flow must be seen as a much coarser measure of the Earth's heat-producing isotopic content. We report here the results of an investigation into the likely variation in terrestrial heat flow over the past 180 Myr.

The empirical relationships between the terrestrial heat flux and the age of oceanic crust or the age of last tectonothermal mobilization of continental crust^{1,2} (Fig. 1) can be used to estimate the global heat flux, provided the age distribution of the crust is known³. Various recent estimates⁴⁻⁶ of the present-day mean terrestrial heat flow fall in the range $81 \pm 3 \text{ mW m}^{-2}$. We present here a probabilistic model of crustal age distributions and heat flow from the present to the early Mesozoic. The model is based on the present-day distribution of crustal ages (see Table 1) and an estimate of the probability that crust produced or tectonothermally mobilized at an earlier time will have survived to the present day.

To determine the age distribution of the oceanic crust at a past time t , oceanic lithosphere which accreted between time t and the present must be subtracted from the present-day distribution, and lithosphere which has been subducted in the same interval must be reinstated; the age of the resurrected lithosphere is, however, uncertain, and must be estimated.

We define an age distribution at time t according to $Q_s(t, \tau) d\tau = Q_0(\tau) d\tau \cdot P_s(t, \tau)$, where $Q_s(t, \tau) d\tau$ is the area of crust of age τ surviving at time t . $Q_0(\tau) d\tau$ is the original area of crust accreted along oceanic ridges at time τ in the time interval $d\tau$, and P_s is a survival probability function giving the probability that crust accreted at time τ survives (has not been subducted) at the later time t . If the total area of oceanic crust is at all times constrained to be equal to the present-day area A_0 , then production and survival can be related by

$$P_s(t, \tau) = 1 - \left[\frac{\int_t^\infty Q_0(\tau) d\tau}{A_0} \right]^{1/(2-1)}$$

in which α is the number of present-day oceanic areas produced by ocean ridge accretion during the past 180 Myr. The mean accretion rate over this period is therefore $\alpha A_0 / 180$. As the area of ocean accreted between times τ and t increases, the survival probability decreases, finally becoming zero. The original area accreted at time τ is estimated from the

Table 1 Percentage of global area represented by crust of indicated age

Ocean		Continent	
Age (Myr)	%	Age (Myr)	%
0-5	3.5	0-65	4.6
5-23	10.9	65-225	5.8
23-38	6.9	225-400	9.2
38-65	10.2	400-600	9.1
65-100	21.2	600-2,500	7.7
100-135	5.4	2,500-3,500	2.0
135-180	3.5		
Total	61.6		38.4

Continental shelves are included as continents of early and late Palaeozoic age.

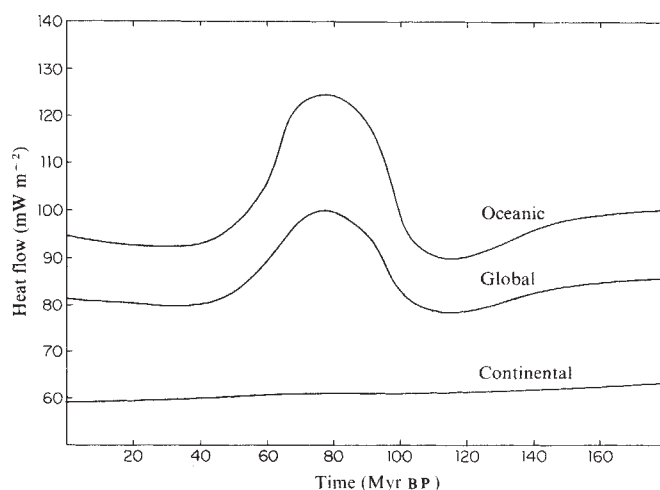
present-day crustal distribution $Q_s(0, \tau) d\tau$ by $Q_o(\tau) d\tau = Q_s(0, \tau) d\tau / P_s(0, \tau)$. As crust older than any now present (>180 Myr) is needed to complete the ocean floor, the mean of $Q_o(\tau)$ from 180 Myr to the present is used.

The form of $P_s(t, \tau)$ is an arbitrary choice. We have tried other smooth, monotonically decreasing functions which satisfy the constraint that the accretion-subduction process yields the present-day area A_0 . We find that the heat flow history is much more strongly dependent on the mean accretion rate, that is the choice of α , than on the particular functional form of P_s .

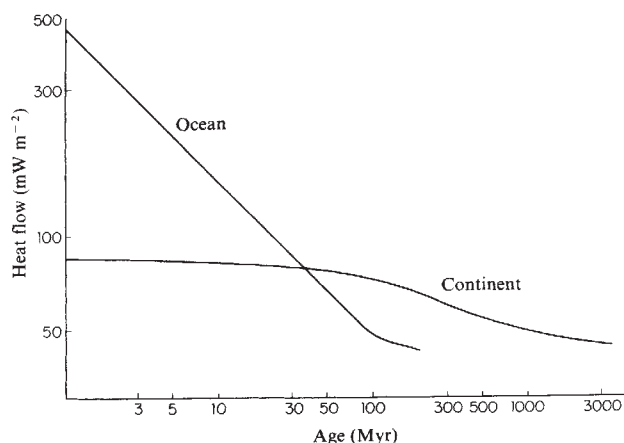
Tectonic processes affecting continents are significantly different from oceanic processes. While there is an increasing probability that old ocean crust will not survive, the same cannot be said for old continental crust. In fact, the survival of extensive areas of continental crust for more than 3,000 Myr testifies to the contrary. Old continental lithosphere is cool, thick, strong and refractory, and moreover tends to be centrally located in continental masses and is thus less frequently affected by plate margin interactions. Accordingly, we have assigned to continental areas a probability of tectonothermal remobilization that decreases exponentially with time since the previous mobilization. In contrast to old crust, recently mobilized continental crust has a high probability of being remobilized, for example in terrains above long-lived subduction zones. Another consideration is that continental mobilizations are not independent of oceanic accretion rates. Intervals of fast accretion must be accompanied by increased subduction with concomitant effects on continental margins. Formally, the calculation of area of continental crust of age τ at time t takes the form $Q_c(t, \tau) d\tau = \beta Q_o(\tau) \exp[-(\tau-t)/t_c] d\tau$, where the constants β and t_c are

evaluated by the requirement that Q_c yield the present-day areas of Cenozoic and Mesozoic terrains. The values of β and t_c so determined are 0.15 and 100 Myr, respectively. The constant t_c is the time constant for the decay of probability of continental remobilizations.

The heat flow in both oceanic and continental regions can thus be calculated from the age distributions $Q_s(t, \tau)$ and $Q_c(t, \tau)$, and the empirical heat flow-age relationships of Fig. 1. An infinite set of possible models obtains in principle from the inherent variability allowed by the relationship between the production rate of oceanic crust and its subsequent probability of survival. All such models yield the present-day distribution of ages (Table 1) and hence the present-day distribution of oceanic heat flow. In application, however, practical constraints such as past spreading rates as deduced from marine magnetic anomalies limit the allowable models to the range $1.5 < \alpha < 3.5$. Our preferred model, shown in Fig. 2 and for which $\alpha = 2.4$, in addition correctly predicts the present-day mean age of subducting crust, 77 Myr. For the range of α cited above, the mean age of subduction ranges from 111 to 60 Myr, thus providing a moderately sensitive guide to the choice of a proper α and a preferred model.

**Fig. 2** Variability of mean oceanic, continental and global heat flow over the past 180 Myr.

The mean accretion rate over the past 180 Myr for this model is such that in that interval 2.4 times the present oceanic area has been produced, of which 1.4 has been subducted. Numerically this mean accretion rate is $1.33\% A_0 \text{ Myr}^{-1}$, slightly above but comparable with estimates of the present-day accretion rate of $0.91\text{--}1.14\% A_0 \text{ Myr}^{-1}$. (The lower value is derived from 'present-day' relative rotation vectors for the world plate system⁷; the upper value is derived from the amount of ocean floor produced in the past 5 Myr.) As shown in Fig. 2, the global heat flux varies between 78 and 101 mW m^{-2} , although for all but 40 Myr the heat flow is within $\pm 5 \text{ mW m}^{-2}$ of the 81 mW m^{-2} present-day flux. The mean of the global heat flow over the 180 Myr interval is 85 mW m^{-2} , slightly above the present-day value. The principal feature of the heat flow history is the peak occurring in the late Cretaceous fast spreading episode; the minimum heat flow occurred in the early Cretaceous just before the opening of the South Atlantic Ocean. Clearly, nearly all of the variation in the global heat flux is due to the oceanic component. This results because of the greater area of ocean, the more extreme variation of heat flow with age in the oceans, and the greater impact of variations in oceanic accretion rate on oceanic heat flow than on continental heat flow.

**Fig. 1** Heat flow versus age relationships for oceans¹ and continents².

We conclude that heat flow over the past 180 Myr has been in the range $78\text{--}101\text{ mW m}^{-2}$. Throughout most of this period the heat flow was close to the present-day value of 81 mW m^{-2} ; the only significant departure was the increase to the peak value of 101 mW m^{-2} during the rapid seafloor spreading of the late Cretaceous. The mean heat flow over the 180 Myr interval is 85 mW m^{-2} , somewhat in excess of the present-day value.

This research was supported in part by the Division of Earth Sciences, NSF, under grant EAR 78-09131.

Received 28 December 1979; accepted 14 March 1980.

1. Parsons, B. & Sclater, J. G. *J. geophys. Res.* **82**, 803–827 (1977).
2. Chapman, D. S. & Furlong, K. *EOS Trans. Am. geophys. Un.* **58**, 1240 (1977).
3. Chapman, D. S. & Pollack, H. N. *Earth planet. Sci. Lett.* **28**, 23–32 (1975).
4. Williams, D. L. & Von Herzen, R. P. *Geology* **2**, 327–328 (1974).
5. Sclater, J. G., Jaupart, C. & Galson, D. *Rev. geophys. Space Phys.* **18**, 269–311 (1980).
6. Davies, G. *Rev. geophys. Space Phys.* (in the press).
7. Minister, J. B. & Jordan, T. H. *J. geophys. Res.* **83**, 5331–5354 (1978).

Cambrian stromatolitic phosphorites from the Georgina Basin, Australia

P. N. Southgate

Research School of Earth Sciences, Australian National University, PO Box 4, Canberra, ACT 2600, Australia

The first details of Phanerozoic stromatolitic phosphorites are reported here. The stromatolites, from the Thornton Limestone in the Georgina Basin, northern Australia are of middle Cambrian age. The carbonates interfinger with and underlie the Beetle Creek Formation which contains Australia's largest known phosphate deposits. Phosphatic stromatolites had previously only been found in Proterozoic rocks, from the middle to upper Riphean Aravalli Group of Rajasthan, India and in the USSR and China¹. It was generally considered that phosphatic stromatolites were unique to the Precambrian^{2,3}. Indeed this unique association had led to hypotheses that during the Precambrian a different set of phosphogenic processes must have existed⁴.

The Georgina Basin is a large epicontinental basin dominated by platform carbonates ranging in age from the early middle Cambrian to the middle Ordovician. On a regional scale the middle Cambrian sequence represents a complex facies mosaic consisting of carbonates, evaporites, phosphorites and terrigenous clastics (Fig. 1). The early middle Cambrian transgression is represented by a basal clastic interval which overlies the Precambrian basement. This interval is overlain by the Thornton Limestone and its lateral facies equivalents. The stromatolites occur in the Thornton Limestone; a carbonate unit consisting of stylolitic vuggy recrystallized dolomite and dolomitic limestone of Ordian to Templetonian age⁵. Minor diastems are common throughout the unit occurring as scalloped surfaces⁶ and as phosphatized hardgrounds which were emergent for brief periods. The phosphatized stromatolites overlie minor dissolution surfaces and hardgrounds.

Although stromatolites occur at various localities within the Georgina Basin⁷, phosphatic stromatolites are only known within 1–4 m thick discontinuous stromatolitic beds, ~10 km south of Riversleigh Station (Fig. 1). Preservation of algal laminae and internal fabric is variable and depends on the type of replacement. Silicification and particularly dolomitization obscure the primary algal structure but where

stromatolites are phosphatic both columnar and laminar form are preserved.

Phosphatic stromatolitic columns up to 4 cm high or phosphatic cryptogalaminite up to 5 cm thick are found overlying minor dissolution surfaces within the Thornton Limestone. Phosphatic laminae up to 2 mm thick occur discontinuously near the basal parts of the stromatolites and immediately overlying minor erosional surfaces within the stromatolitic bioherms. Chert-replaced laminae are not related

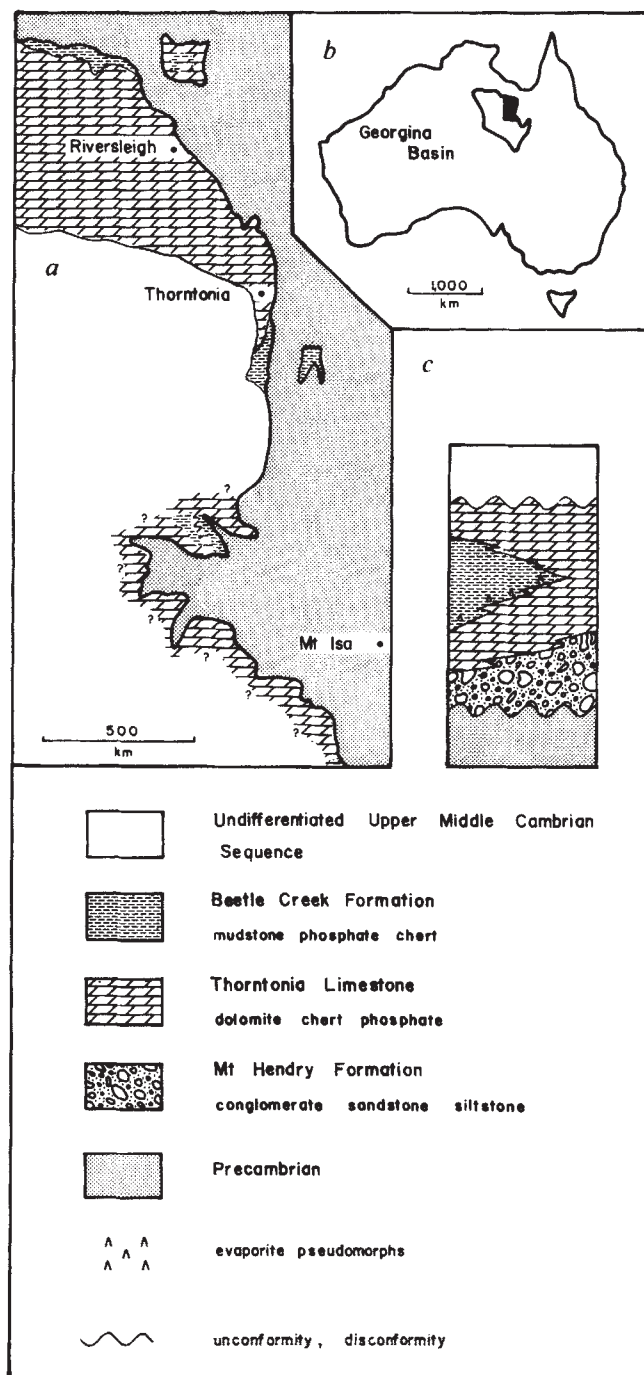


Fig. 1 a, map showing the northeastern margin of the Georgina Basin and the close lateral relationship between outcrop of Thornton Limestone and Beetle Creek Formation. b, Location map for the Georgina Basin, the north-east portion shown in a. is shaded. c, Generalized stratigraphic column for the lower middle Cambrian sequence of the northeastern Georgina Basin.

to minor erosional surfaces or diastems and occur randomly throughout the dolomitized parts of the stromatolites. Phosphatic stromatolites, consisting of not less than 90% colophane are generally thinnest at the base and thicken upwards. Elongate inclined columns occur as LLH-S and SH forms⁸. Columns overlie a phosphatic planar algal mat characterized by fine laminoid fenestrae (Fig. 2). The basal 0.5–2 cm of columns consist of phosphatic mat with laminae parallel to the underlying planar mat. These basal parts form low ridges which are wrapped around and overlain by a convex-upward lamination defining SH-C and V growth forms. Similar ridge and rill structures have been described from the lower intertidal zone at Shark Bay⁹ and form when the planar mat is partly eroded. Ridges form where mat remains, and rills form where the mat is eroded. Later mat growth occurs preferentially on ridge tops and sides resulting in the columnar forms. Inclined and vertical stromatolites, circular in plan, occur both as single columns and laterally linked forms in the Thornton Limestone. Branching is present in both elongate and circular stromatolites.

Fine laminoid irregular fenestrae, now filled with dolomite occur in both columns and planar mat. Some enlarged fenestrae have several straight edges with angular relationships between the edges. As well as enlarged and fine laminoid irregular fenestrae there are slender slit-like lenticular, blocky and twinned crystal shapes and dolomite rhombs within the phosphatic columns and mat. The elongate crystal shapes are in vertical to sub-horizontal orientations. Some crystal shapes preserve remnants of a right angle cleavage and twinned crystals are characterized by re-entrant angles suggestive of former gypsum twins within the algal mats. Where angular relationships can be shown not to be due to early dolomite growth enlarged fenestrae are taken to indicate early sulphate mineral growth in fenestral voids, the displacive and/or replacive sulphate growth enlarge the fenestrae once the initial void has been filled (B. Radke, personal communication).

Whereas columnar stromatolites are phosphatic, intercolumn areas are non phosphatic, consisting of equant recrystallized dolomite with limonite present along grain boundaries. No phosphate replacement of carbonate grains or cement has been seen in intercolumn areas; the only phosphate present occurs as reworked grains of phosphatic skeletal sponge spicules and trilobite debris, as well as ovules, and internally laminate clasts of eroded phosphatic algal mat. Some skeletal grains of uncertain origin are outlined by micritic envelopes which enclose equant recrystallized dolomite. Phosphatic clasts and ovules are angular to well rounded with carbonate inclusions. Ghosted, non-phosphatic peloids are also present between columns as are sporadic glauconite grains. Ghost textures in recrystallized intercolumnar areas indicate that infills were probably either skeletal grainstone or packstone.

As shown in Fig. 1, the Thornton Limestone interfingers with the Beetle Creek Formation which consists of siltstone, phosphate and chert. The phosphate deposits of the Georgina Basin consist mostly of grainstone phosphate with minor microsporite^{7,10}. Consequently the locations of phosphate deposits mark sedimentary traps in which grains have accumulated. It follows that these grains may have been derived from a nearby source where phosphatization took place. The recognition of phosphatic stromatolites within the contemporaneous Thornton Limestone indicates that *in situ* phosphatization indeed took place in this limestone unit. Erosion of stromatolites is one possible source of phosphatic grains. Phosphatized hardgrounds (P.N.S. in preparation) have also been found in the Thornton Limestone further indicating the potential of this formation as a possible source for the grains of phosphate.

There are many similarities between Thornton Limestone phosphatic stromatolites and those reported from Rajasthan, India^{2,3}. *Collenia* LLH, cryptozoan SH and oncolitic SS forms as well as *Conophyton* are documented from Indian

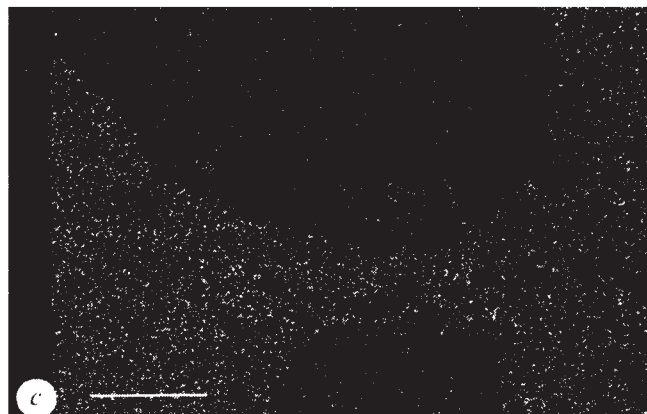
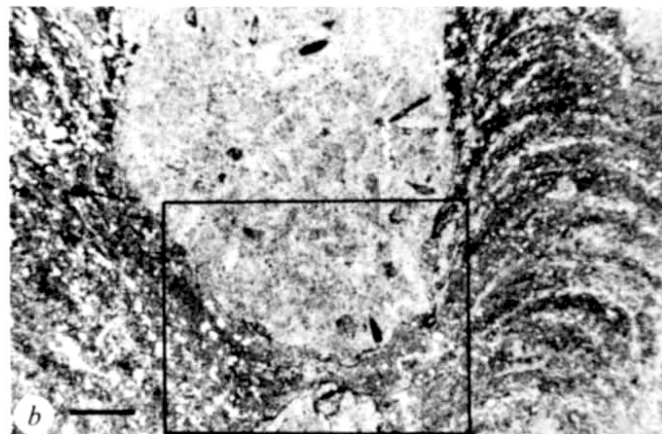


Fig. 2 *a*, Elongate, inclined LLH-S and SH columns and cryptalgalaminite. Dark areas within the phosphatic stromatolites are dolomite filled fenestrae. White specks within intercolumnar areas of dark grey dolomite are phosphate grains. Scale bar, 1 cm. *b*, Transmitted light photomicrograph of an intercolumnar area surrounded by phosphatic columnar stromatolites. Light areas outlining the algal laminations are fine laminoid fenestrae. Several phosphate grains are present in the intercolumnar area (see *c*). Scale bar, 1 mm. *c*, X-ray mapping photograph of distribution of phosphate within the area outlined in *b*. Note the concentration of phosphate (white specks) within the algal laminate parts. Also the presence of several phosphate grains within the intercolumnar area, particularly in the middle centre and middle left of the photograph. Scale bar, 1 mm.

occurrences^{2,3}. Thornton Limestone stromatolites consist of LLH-S and SH-V and C growth forms. Phosphatic oncolites have not been found in the Thornton Limestone; Conophyton is not known from the Phanerozoic. The Indian and Australian examples are characterized by rapid variations in bioherm thickness along the strike; in the same bioherm completely, partially and non-phosphatic stromatolites are found only a few metres apart. Both occurrences are characterized by a marked localization of phosphate; only algal laminated rocks are phosphatic and intercolumnar areas are always non phosphatic, apart from scattered reworked phosphate grains and clasts. No reason is given for the variations in phosphate content of Indian stromatolites but those from the Georgina Basin seem to be related to underlying or adjacent dissolution surfaces or diastems.

The presence of scalloped surfaces, ridge and rill structures, inclined columns, ? sulphate pseudomorphs and enlarged fenestrae are all consistent with a shallow water to semi-emergent environment of deposition for Thornton Limestone phosphatic stromatolites. Furthermore the occurrence of fine laminoid fenestrae indicates that the phosphatic algal mat may be smooth mat, a mat type characteristic of the lower intertidal zone⁹. Similarly, Indian examples are thought to form in shallow water conditions, possibly in lagoons and on tidal flats^{2,3}.

The association between phosphatic stromatolites and features indicative of semi-emergent conditions of sedimentation are particularly significant. The reworked clasts of phosphatic cryptogaminites clearly show that the stromatolites were either originally phosphatic or phosphatized as a syn-sedimentary event. The close spatial association between phosphatic stromatolites and scalloped or dissolution surfaces is also important, for even though the stromatolite bioherm may be up to 4 m thick, only where algal growth is adjacent to an erosion surface are the stromatolites phosphatic. This relationship suggests that very specific physical and/or chemical conditions are necessary for the formation of phosphatic stromatolites. The nature of these conditions is still being investigated: however, the irregular relief provided by dissolution surfaces would have resulted in the formation of intertidal pools. Stromatolites growing in these pools may have been periodically subjected to unusual or stressed chemical conditions, as both the water level and temperature varied and it may have been this stressed environment which created the appropriate physicochemical conditions for the formation of phosphatic stromatolites.

I conclude that the Georgina Basin phosphatic stromatolites and associated sedimentary structures show clear evidence of having formed in very shallow to semi-emergent environments and, furthermore, indicate that phosphatic stromatolites are not a uniquely Precambrian phenomenon.

I thank P. J. Cook of ANU, M. Muir and B. Radke of BMR for comments and the staffs of ANU and BMR for their assistance in various aspects of the research programme. This paper is a contribution to ICGCP Project 156.

Late Tertiary hominoids and human origins

Henry M. McHenry

Department of Anthropology, University of California, Davis, California 95616

Robert S. Corruccini

Department of Anthropology, Southern Illinois University, Carbondale, Illinois 62901

There are two principal hypotheses about the time of the emergence of the Hominidae, the family that includes man: first, the hominid lineage emerged before 16 Myr ago¹⁻⁴; or second, living apes and man are so similar genetically that they must have diverged later⁵, perhaps as late as 5 Myr ago^{6,7}. The first hypothesis is supported primarily by fossil evidence, whereas the second is championed by advocates of the molecular clock^{6,7}. Hominid fossils are abundant back to 3.7 Myr (*Australopithecus afarensis*)⁸ but are rare before that. *Ramapithecus* (16–8 Myr) was once classified as a member of Hominidae, but recent discoveries and interpretations show that it should be placed in its own family, *Ramapithecidae*^{9,10}. Two fossil specimens classified as members of Hominidae predate *A. afarensis*, however: a lower molar from Lukeino in central Kenya dating to 6.5 Myr (KNM-LN 335)¹¹ and a mandibular fragment from Lothagam Hill in north Kenya dated at 5–5.5 Myr (KNM-LT 329)¹². Does their morphology justify classification as the first members of Hominidae? Their geological age is relatively secure. The Lukeino Formation which is up to 130 m thick is bracketed by K/A dates of 7–5.4 Myr¹¹. The deposits from which the Lothagam Hill mandible derives are disconformably overlain by an intrusive basalt sill with a K/A date of 3.7 Myr and are associated with mammalian fauna that yields a biostratigraphical age of 5–5.5 Myr^{12,13}. We report here a morphological study which indicates that the Lukeino molar has cusp proportions similar to the modern chimpanzee and the Lothagam Hill mandible is morphologically intermediate between modern pongids and *A. afarensis*.

Because taxonomic assessment is difficult with small fragments of this sort, we have sought affinities by a series of analyses using multiple measurements, including the basal diameters of the five cusps, occlusal length, cervical length and breadth, trigonid and talonid breadths, diagonal length of the crown, and crown height for both the first and second molars, plus breadth and depth of the mandible under the second molar and cervical lengths and breadths of adjacent teeth^{14–18}. These measurements are defined so as to reflect features of importance as indicated by ontogenetic and functional considerations that are independent of wear. The same measurements are recorded for 50 specimens each of *Homo sapiens* (multi-ethnic), *Pan troglodytes*, *Gorilla gorilla*, and *Pongo pygmaeus*, 22 *Pan paniscus*, 17 *Ramapithecus*, 19 *Sivapithecus*, 7 *Proconsul africanus*, 15 *P. nyanzae*, 7 *P. major*, 3 *Australopithecus afarensis*, 7 *A. africanus*, 11 *A. robustus*, 7 *A. boisei*, 17 early *Homo*, and 9 *H. erectus*. We used Penrose's method¹⁹ to calculate morphometrical distance due to size and

Received 18 January; accepted 28 March 1980.

- Bushinskii, G. I. in *Delta and Shallow Marine Deposits* (ed. Van Straaten, L. M. J. U.) 62–70 (Elsevier, Amsterdam, 1964).
- Bushinskii, G. I. *Old phosphorites of Asia and their Origin* (translation from *Trans. Akad. Nauk SSSR, Moskva*, No. 149) (Jerusalem, Israel Programme for Scientific Translation, 1969).
- Banerjee, D. M. *Bull. geol. Soc. Am.* **82**, 2319–2330 (1971).
- Sant, V. N. in *Proterozoic and Cambrian Phosphorites* Rept on 1st Int. Meeting of ICGP, Project 156 (eds Cook, P. J. & Shergold, J. H.) 39–40 (ANU, Canberra, 1979).
- Burnett, W. C. & Sheldon, R. P. (eds) *Report on the Marine Phosphatic Sediments*, 43 (Workshop, Honolulu Hawaii, East-West Centre 1979).
- Shergold, J. H. & Druce, E. C. *Proc. 3rd Aust. Geol. Conv.*, Townsville (1978).
- Read, J. F. & Grover, G. A. *J. Sed. Petrol.* **47**, 956–972 (1977).
- de Keyser, F. & Cook, P. J. *Bur. Miner. Res. Bull.* **138**, 79 (1972).
- Logan, B. W., Rezak, R. & Ginsburg, R. N. *J. Geol.* **72**, 68–83 (1964).
- Logan, B. W., Hoffman, P. & Gebelein, C. D. in *Evolution and Diagenesis of Quaternary Carbonate Sequences, Shark Bay, Western Australia*, 190–194 (AAPG Mem 22, 1974).
- Russell, R. T. & Trueman, N. A. *Econ. Geol.* **66**, 1186–1214 (1971).

Table 1 Size and shape distances of hominoids from the Lukeino fossil

Taxon compared to Lukeino	Penrose distance		Mahalanobis D	
	Size	Shape	Raw	Regression-adjusted
<i>Pan troglodytes</i>	0.53	0.49	24.21	9.95
<i>P. paniscus</i>	1.35	0.70	32.02	11.83
<i>Australopithecus afarensis</i>	3.00	1.36	32.06	13.44
<i>A. africanus</i>	3.38	1.35	46.07	13.95
<i>A. robustus</i>	4.22	1.39	60.72	12.50
<i>A. boisei</i>	6.16	1.62	75.48	12.31
<i>Homo</i> (fossil and recent)	1.92	0.99	43.72	14.42

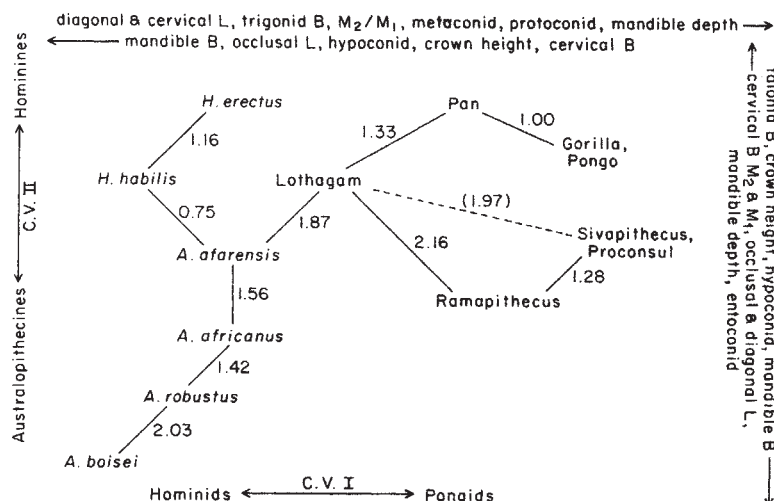


Fig. 1 Results of the canonical variates analysis of 16 size-standardized dimensions of the first molar and mandible. Traits having high correlations with canonical variate I (abscissa) and II (ordinate) are indicated in the margins with the direction of their relative increase. Taxa are indicated by their sequential arrangement when projected onto the two variates; superimposed distance between samples are direct linear shape z-score distances which are highly correlated with Mahalanobis D distances ($r=0.88$).

shape differences among specimens. We also performed canonical variates analyses²⁰ for which we used size-standardized data²¹. Size standardization was done by normalizing the measurements, forming a standard size variable which was the average normalized measurement magnitude for each case, regressing the standardized measurements against this size variable, and using the pooled within-species regression coefficient to adjust the standardized measurements to their residual from the size regression²¹.

When the data are size-standardized in this way the isolated molar from Lukeino becomes metrically almost indistinguishable from modern pongids. It shares no salient hominid apomorphies (shared derived traits) or even the incipient development of such traits in the M_2 measurements. The smallest Penrose distance coefficient is with *P. troglodytes* (0.49) which is smaller than any other hominoid (Table 1). Other measures of distance give the same result. None of the results affords a basis for separation of the Lukeino molar from the genus *Pan*, but we do not advocate this classification because the close metrical similarity may reflect only shared primitive characters and there are other features of the fossil not measured by our study which differ from *Pan*¹².

The first molar and adjacent mandibular parts of the fossil from Lothagam Hill seem to have an overall morphological pattern intermediate between pongids and hominids. Figure 1 presents the results of the canonical variates analysis based on M_1 and mandibular traits. The major canonical variate separates hominids from pongids with Lothagam Hill intermediate between them. The morphological pattern characterizing hominids along this axis includes square, relatively short molars with centrally situated hyponulid, reduced trigonid breadth and protoconid and metaconid diameters, enlarged M_1 compared to M_2 , shallow and thick mandibular corpus, expanded occlusal foveal length and crown breadth, large hypoconid, and high crown. The second canonical variate separates the australopithecines (with *A. boisei* at the far extreme) from the hominines. The extreme specialization of the *A. boisei* molars is reflected by the great distance separating them from other hominids. The distances shown in Fig. 1 are the direct linear z-score distances which are highly correlated with the Mahalanobis D distances²⁰ ($r=0.88$). Measured in this way the Lothagam Hill specimen is intermediate between *Pan* and the earliest australopithecine, although closer to *Pan troglodytes* than to either *P. paniscus* or *A. afarensis*. It is well removed from *Ramapithecus*.

These results show that the morphology of the Lukeino molar does not necessarily justify its inclusion in the family Hominidae but the Lothagam Hill mandible does resemble later hominids in some ways. However, phylogenetic affinity depends not on a simple demonstration of degrees of similarity or difference, but on the identification of specific shared derived features independent of shared primitive traits. Among

our suite of metrical traits the Lukeino molar shows no derived features in common with *Australopithecus*. The fossil is within the sample confidence limits of *Pan troglodytes* in almost all variables, falling close to the mean for most of them. Other features such as thick enamel are not unique to Hominidae, but are present in *Gigantopithecus*, *Sivapithecus*, *Ramapithecus* and, to some extent, *Pongo*. Without further evidence it seems justifiable only to classify the Lukeino molar as Hominoidea indet. The Lothagam Hill fossil, on the other hand, does share some derived traits unique to known early members of Hominidae: relatively short occlusal length of M_1 , relatively small M_1 entoconid diameter, and relatively shallow mandibular depth under M_2 . It lacks several other derived features which *A. afarensis* shares with all other hominids, such as relatively large first molar cervical, and talonid breadths, relatively high crowned M_1 and relatively broad mandibular corpus under M_2 .

Therefore, we recommend the Lothagam Hill specimen not be placed in *A. afarensis*, but left as Hominidae indet. It seems, therefore, that the first known member of the Hominidae is about 5 Myr old. This does not rule out the possibility of an early divergence of our evolutionary lineage, but does negate palaeontological objections to the late divergence hypothesis.

We thank C. K. Brain, E. Vrba and staff of the Transvaal Museum, P. V. Tobias and staff of the Department of Anatomy, University of Witwatersrand, R. E. F. Leakey, M. D. Leakey and B. A. Ogot and staff of TILLMIAP, I. Tekkaya, M. Kretzoi, G. H. R. von Koenigswald, D. R. Pilbeam and F. C. Howell for advice and permission to examine the fossils, D. F. E. T. van den Audenaerde and the staff of the Musée Royale de l'Afrique Centrale, for use of material, and L. J. McHenry and P. Andrews for advice and assistance. This work was supported in part by the Committee on Research, University of California, Davis.

Received 21 September 1979; accepted 20 March 1980.

1. Simons, E. L. *Primate Evolution* (Macmillan, New York, 1972).
2. Simons, E. L. *J. Hum. Evol.* **5**, 511–528 (1976).
3. Simons, E. L. *Scient. Am.* **236**(5), 28–35 (1977).
4. Pilbeam, D. *The Ascent of Man* (Macmillan, New York, 1972).
5. Washburn, S. L. & Moore, R. *Ape into Man* (Little & Brown, Boston, 1974).
6. Sarich, V. M. & Wilson, A. C. *Science* **158**, 1200–1203 (1967).
7. Sarich, V. M. & Cronin, J. E. in *Molecular Anthropology* (eds Goodman, M. & Tashian, R. E.) (Plenum, New York, 1977).
8. Johanson, D. C. & White, T. D. *Science* **202**, 321–330 (1979).
9. Pilbeam, D. A. *Rev. Anthropol.* **8**, 333–352 (1979).
10. Pilbeam, D. *Nature* **270**, 689–695 (1977).
11. Pickford, M. *Nature* **256**, 279 (1975).
12. Patterson, B., Behrensmeyer, A. K. & Sill, W. D. *Nature* **226**, 918–921 (1970).
13. Behrensmeyer, A. K. in *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y. et al.) (Univ. Chicago, 1976).
14. Corruccini, R. S. thesis, Univ. California (1975).
15. Corruccini, R. S. *Z. Morph. Anthropol.* **68**, 14–25 (1977).
16. Corruccini, R. S. *J. dent. Res.* **56**, 190 (1977).
17. Corruccini, R. S. *J. dent. Res.* **56**, 1093–1096 (1977).
18. Corruccini, R. S. *Archs oral Biol.* **23**, 491–494 (1978).
19. Penrose, L. S. *Ann. Eugen.* **18**, 337–343 (1954).
20. Blackith, R. E. & Reyment, R. A. *Multivariate Morphometrics* (Academic, London, 1971).
21. Corruccini, R. S. *Homo* **28**, 222–226 (1978).

Independent effects of the pineal and a bacterial pyrogen in behavioural thermoregulation in lizards

Bruce T. Firth*, Charles L. Ralph*
& Thomas J. Boardman ‡

*Department of Zoology and Entomology, and ‡ Department of Statistics, Colorado State University, Fort Collins, Colorado 80523

The pineal complex of lizards is comprised of an extracranial photoreceptive structure known as the parietal eye, and an intracranial pineal organ which is homologous to the pineal gland of birds and mammals. Studies have shown that removing the parietal eye^{1,2} or severing the parietal nerve³ causes lizards to select higher temperatures when allowed to thermoregulate behaviourally in thermal or photothermal laboratory gradients. Although comparable studies involving removal of the lizard pineal organ have not previously been attempted, field data indicate that pinealectomy may have an antagonistic effect to parietectomy⁴. We present evidence here which shows that (1) following pinealectomy, collared lizards (*Crotaphytus collaris*) behaviourally select or prefer lower temperatures than their controls in thermal laboratory gradients, and (2) the effect of surgical treatment is independent of the effects of a behavioural fever-inducing substance⁵ which elevates by a fixed amount the environmental temperatures selected.

Eighteen collared lizards were divided into three treatment groups of six individuals each, all groups being essentially equivalent in body mass and sex ratio. One group was pinealectomised (P), one sham-pinealectomised (S) and the other group remained intact (I). Pinealectomy was accomplished by drilling through to the meninges an area with its anterior border 2 mm posterior to the interparietal scale. A small slit was made in the meninges and the pineal organ removed with watchmaker forceps. The wound was packed with Gelfoam and later sealed with dental cement. Sham-pinealectomy was achieved in the same way with the omission of the pineal removal step. Effectiveness of surgery was confirmed later by histology. In early July, 6 weeks after surgery, the animals were released randomly into individual sand-covered runways along which

† Present address: Department of Zoology, University of New England, Armidale, NSW 2351, Australia.

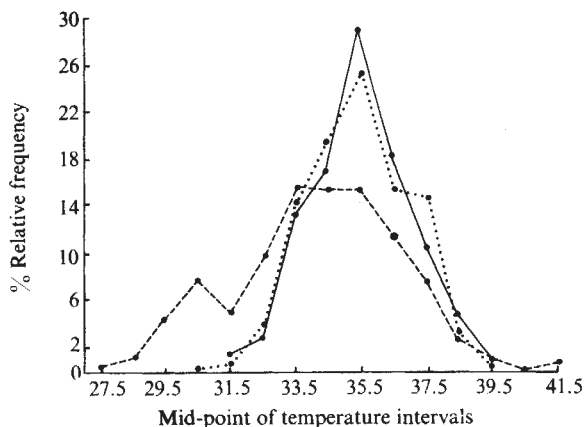


Fig. 1 Percentage relative frequency of temperatures selected by intact (—), sham-pinealectomised (···) and pinealectomised (---) *C. collaris* before pyrogen or saline injection. Temperatures on the x axis represent mid-points of 1 °C class intervals.

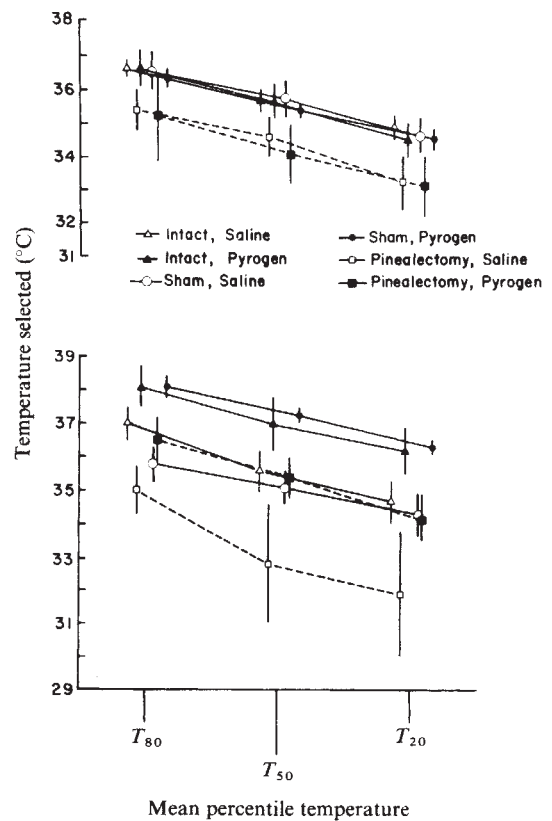


Fig. 2 Thermal selection in laboratory gradients at night by pinealectomised (P), sham-pinealectomised (S) and intact (I) *C. collaris* injected with either bacterial pyrogen or the saline vehicle. Means and standard errors (vertical lines) are compiled from two experiments carried out 5 days apart, and represent for each treatment ($n = 6$) the average of the median temperature (50th percentile or T_{50}), and temperatures around the median, that is, at the 80th percentile and 20th percentile (T_{80} and T_{20}), respectively. The temperatures at the three percentiles were obtained by ranking the 50 temperatures recorded for each animal between 1800 h and 0600 h. Thus, the temperature at T_{80} was the 10th highest temperature, that at T_{50} the 25th highest and that at T_{20} the 10th lowest. The upper plot shows thermal behaviour in the six groups of lizards the night before injection with either saline or pyrogen. The only significant factor affecting thermal selection was pinealectomy, which depressed the preferred temperature at all three ranked levels (two-way ANOVA; T_{80} , $P < 0.05$; T_{50} , $P < 0.01$; T_{20} , $P < 0.01$). A similar two-way ANOVA following the injection of saline or pyrogen (lower plot) showed a significant effect due to this factor (T_{80} , $P < 0.01$; T_{50} , $P < 0.02$; T_{20} , $P < 0.05$), in addition to a significant effect due to surgical treatment (T_{80} , $P < 0.01$; T_{50} , $P < 0.05$; T_{20} , $P < 0.05$). The injection of saline in the three treatment groups (P, S, I) did not significantly alter the pattern observed before injection. However, the injection of pyrogen caused a significant fever of 1–2.5 °C in the three treatment groups. Despite these independent effects, there was no significant interaction (T_{80} , $P < 0.5$; T_{50} , $P < 0.8$; T_{20} , $P < 0.9$) between surgical and injection treatments.

existed a thermal gradient of 12–48 °C. A 12-h light/12-h dark photoperiod (12 L:D, L 0600 h–1800 h) was imposed by overhead fluorescent lights controlled by a timer. The hot end of the gradient was provided by incandescent lamps only during the light phase and by heat tape only during the dark phase. The cold end was maintained by cold water pumped through copper tubing. As skin temperature is thought to be an important regulated variable in lizard thermoregulation⁶, only skin surface temperatures of individual lizards were recorded every 15 min from 36-gauge thermocouples taped to the base of the tail and connected to a Honeywell 112 multichannel temperature recorder.

The lizards remained in their respective runways for 10 days before the temperature measurements reported here. They were fed crickets and mealworms at the same time daily. Between 0900 h and 0930 h on day 12, half of each treatment group was injected intraperitoneally with pyrogen, a substance initiating behavioural fever⁵, consisting of a 0.5 ml suspension of 2×10^9 dead *Aeromonas hydrophila* bacteria in a vehicle of 0.85% sterile saline. The remaining half was injected with the vehicle only. Because preliminary studies showed that pinealectomy had a greater effect on thermal behaviour during the scotophase, the 11th night was chosen as the pre-injection period and the 12th night as the post-injection period. The experiment was repeated 5 days later, using the same animals and the same procedure, except that the lizards previously injected with pyrogen were injected with the vehicle and vice versa.

A frequency distribution of the temperatures selected by P, S and I lizards before injection of either saline or pyrogen reveals that P animals preferred lower temperatures than controls and that temperatures were more broadly distributed than for controls (Fig. 1). These results indicate an impaired ability to thermoregulate precisely after pinealectomy. A detailed analysis of median and paramedian temperatures selected by lizards before and after injection of bacterial pyrogen (Fig. 2) shows that the preferred temperatures of all surgically treated and intact groups were elevated by a fixed amount. However, no significant interaction was observed between pinealectomy and pyrogen treatment, indicating that the pineal organ of *C. collaris* does not participate in behavioural fever.

Previous studies have shown that pinealectomy in birds and mammals can alter thermoregulatory function by inducing hyperthermia, hypothermia and disruption related to temporal and/or photic cues⁷⁻¹⁰. The present data indicate that behavioural thermoregulation of an ectotherm may similarly be influenced by the pineal, perhaps by altering the sensitivity or set point of a central thermostat. As it is generally regarded that this thermostat resides in the hypothalamus of both endotherms¹¹ and ectotherms^{12,13}, the thermoregulatory effects of pinealectomy may well be related to changes in hypothalamic input. Evidence for such an hypothesis is provided by (1) the anatomical and functional relationship between the pineal organ and the hypothalamus in several vertebrate groups¹⁴, and (2) our observation that P lizards increase the range of temperatures behaviourally selected (Fig. 1), a finding similar to that shown for the thermoregulatory shuttling behaviour following preoptic hypothalamic lesions in the lizard *Dipsosaurus dorsalis*¹⁵. Our results further indicate that behavioural fever, which is believed to be manifested by an elevation in the set point of the central thermostat¹⁶, is triggered by an action of pyrogen on centres other than those affected by the pineal.

This work was supported by NSF grant PCM 75-18187. We thank Ingrid Belan for transcribing data, J. Starkey for technical assistance, and Evryll Swanson and J. Collins for advice in preparing bacterial pyrogen. H. Heatwole, M. Kavaliers, W. Gern, C. R. Tracy and J. Turner critically reviewed the manuscript.

Received 2 January; accepted 21 February 1980.

- Hutchinson, V. H. & Kosh, R. J. *Oecologia* **16**, 173-177 (1974).
- Roth, J. J. & Ralph, C. L. *J. exp. Zool.* **198**, 17-28 (1976).
- Engbretson, G. A. & Hutchinson, V. H. *J. exp. Zool.* **198**, 29-38 (1976).
- Stebbins, R. C. *Copeia* **1960**, 276-283 (1960).
- Vaughn, L. K., Bernheim, H. A. & Kluger, M. J. *Nature* **252**, 473-474 (1974).
- Barber, B. J. & Crawford, E. C. *Jr Physiol. Zool.* **52**, 250-263 (1979).
- Binkley, S., Kluth, E. & Menaker, M. *Science* **174**, 311-314 (1971).
- Cogburn, L. A., Harrison, P. C. & Brown, D. E. *Proc. Soc. exp. Biol. Med.* **153**, 197-201 (1976).
- Spencer, F., Shirer, H. W. & Yochim, J. M. *Am. J. Physiol.* **231**, 355-360 (1976).
- John, T. M., Itoh, S. & George, J. C. *Horm. Res.* **9**, 41-56 (1978).
- Hammel, H. T. *A. Rev. Physiol.* **30**, 641-710 (1968).
- Crawshaw, L. I. & Hammel, H. T. in *The Pharmacology of Thermoregulation* (eds Schonbaum, E. & Lomax, P.) 142-145 (Karger, Basel, 1973).
- Hammel, H. T., Crawshaw, L. I. & Cabanac, H. P. in *The Pharmacology of Thermoregulation* (eds Schonbaum, E. & Lomax, P.) 124-141 (Karger, Basel, 1973).
- Ralph, C. L., Firth, B. T. & Turner, J. S. *Am. Zool.* **19**, 273-293 (1979).
- Berk, M. L. & Heath, J. E. *J. therm. Biol.* **1**, 65-78 (1975).
- Kluger, M. *Fedn Proc.* **38**, 30-34 (1979).

Avian flocking in the presence of a predator

Thomas Caraco*, Steven Martindale & H. Ronald Pulliam†

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

Although there are several possible advantages of flocking¹⁻³, many authors suggest that birds forage in groups to reduce the risk of predation (see citations in ref. 4). One version of the 'many eyes' hypothesis proposes that flocking allows individuals to spend less time scanning for predators and more time feeding^{5,6}. However, flocking may also cause individuals to lose time and energy in fighting one another. The way a bird divides its time among these activities, its time budget, may depend on variables governing foraging requirements and the chance of predation⁷. As one such variable is the frequency of attacks by predators⁸, we flew a trained hawk over flocks of granivorous yellow-eyed juncos (*Junco phaeonotus*) to compare time budgets in the presence and absence of a predator. We found that time budgets changed after the predator was introduced and also that flock size increased in the presence of the predator.

Previous studies indicate that individual yellow-eyed juncos scan for predators less often as flock size increases, while both feeding time and aggressive behaviour increase simultaneously⁷. The proportion of time an individual spends in aggressive behaviour also varies with temperature and food availability. Dominant birds have less time to defend foraging areas when they must spend more time feeding because of low ambient temperature or low seed density. As foraging time requirements increasingly constrain aggression, lower ranking birds are not so quickly evicted from better foraging areas, and flock size increases⁷. Thus, some of the variations in foraging flock size can be explained by the relative advantages and disadvantages of flocking in terms of time budgets⁹.

If the frequency of predator attacks increases, an individual might be able to increase its survivorship by scanning more often, as long as feeding time is sufficient to prevent starvation. Larger flocks might form if increased scanning entails a reduction in aggression, if grouping *per se* enhances the individual's probability of avoiding predation^{4,10}, or if only larger flocks allow sufficient feeding time at high attack frequencies⁵.

We released a trained Harris hawk (*Parabuteo unicinctus*) in our study area for 2 h (1500-1700 h) on each of 3 days during 2 weeks in February 1978. The hawk perched in tall trees and swooped down over junco flocks to obtain a food reward from its trainer. The Harris hawk was ideal, because it did not actually catch juncos, but it did appear to 'attack'. Harris hawks seldom hunt small birds, and the juncos easily outmanoeuvred the experimental hawk (compare ref. 11).

Time budgets were sampled by observing randomly selected focal birds. At 15-s intervals we recorded the behaviour being exhibited by the bird under observation and categorized the behaviour as: (1) scanning for predators; (2) feeding (search for and handling of seeds); or (3) interference (actual aggression or movement away from nearby birds). These three categories are mutually exclusive and encompass all the activities of the birds

* Present address: Department of Biology, University of Rochester, Rochester, New York New York 14627.

† Present address: H. S. Colton Research Center, Museum of Northern Arizona, Flagstaff, Arizona 86001.

as long as they are members of foraging groups. The categories are easily distinguishable when observing juncos. For each flock size, we pooled the data from all individuals to obtain large samples and then calculated time budgets as the proportion of observations associated with each behavioural category. Data were compared with those obtained in the same area, at the same temperature, during a previous year⁷.

We next recorded the rate at which individuals scanned for predators (as a function of group size) when the hawk was present. Data were computed as scans per min and compared with scanning rates observed under normal predator attack frequency. We also recorded the size of all foraging flocks observed in the study area during 2-h periods before and after the hawk was released. Temperature did not vary more than 2 °C among sampling periods, so that any flock size variation could not be attributed to a change in temperature-dependent constraints on aggression.

Table 1 Time budgets with and without hawk in the study area

Flock size	1	3-4	6-7	
Hawk absent	T_S	0.30	0.13	0.07
	T_F	0.70	0.77	0.85
	T_I	0.00	0.10	0.08
	n	159	162	149
				$\bar{G} = 3.93 \pm 0.86$
Hawk present	T_S	0.57	0.27	0.33
	T_F	0.43	0.69	0.60
	T_I	0.00	0.04	0.07
	n	79	52	162
				$\bar{G} = 7.33 \pm 1.6$

T_S , proportion of time spent scanning for predators; T_F , proportion of time spent feeding; T_I , proportion of time spent in interference. n , Sample size, and $\bar{G}$ is mean flock size $\pm$ standard error.

Table 1 summarizes the time budget data, with and without the hawk present, for flocks of 1, 3 or 4, and 6 or 7 birds. In both conditions, solitaires scanned more often than individuals foraging with other juncos. When the hawk was present, juncos spent significantly more time scanning as solitaires ($P < 0.005$, likelihood ratio test), in flocks of 3 or 4 ($P < 0.025$) and in flocks of 6 or 7 ($P < 0.005$). Feeding time was necessarily significantly less for solitaires (since scanning was greater) and also was significantly less for flocks of 6 or 7 ($P < 0.005$). The effect on feeding time alone was not significant in flocks of 3 or 4, although interference decreased as scanning increased. Mean flock size before the hawk's release was 3.93, compared with 7.33 when the hawk was present. This difference is statistically significant ($t_{46} = 2.03$, $P < 0.025$).

Whether the hawk was present or absent, the rate at which an individual scanned for predators decreased significantly with increasing flock size. If we let $y_1 = \log_{10}$ (scans per min per bird) with the hawk absent, and $y_2 = \log_{10}$ (scans per min per bird) with the hawk present, from our previous report⁷ without a hawk $y_1 = 1.047 - 0.399 \log_{10}$ (group size), $r = -0.92$, and with the hawk present we find that $y_2 = 1.48 - 0.38 \log_{10}$ (group size), $r = -0.72$. Analysis of covariance indicates that the slopes do not differ significantly ($F_{1,25} = 0.03$, not significant); however, the weighted means (therefore, the intercepts) do differ significantly ($F_{1,26} = 60.2$, $P < 0.005$). As flock size increased, there was a reduction in individual scanning rate under both treatments, but for any given flock size individuals scanned more often with the hawk present.

As the hawk's presence increased flock size, the relative advantages of flocking apparently increase for juncos as the frequency of predator attacks increases. The hawk's presence tended to increase scanning at the expense of feeding time more than interference. However, the feeding time of an individual foraging with others, compared to a solitary's feeding time,

showed a greater relative increase when the hawk was present. We interpret this as an indication that an individual can acquire a greater advantage with respect to feeding by joining a group when predator attacks are more frequent. As we have shown previously⁷, the slope of the regression of individual scanning rate on group size suggests that an attack is more likely to be detected as flock size increases, so that larger flocks offer better protection. Furthermore, a hawk cannot capture more than one bird per attack, so an individual's probability of being the victim of successful attack decreases as flock size increases. As long as predators do not concentrate all their efforts on flocks, the relative advantages of flocking to an individual junco apparently increase with attack frequency. Predator attack frequency is then another ecological variable affecting junco flock size and its impact is mediated, at least in part, through time budgeting variation.

We thank T. S. Whittam, J. Todd, Y. Cox and B. Bristow (Arizona Game and Fish Department) for assistance. This work was supported by a US NSF grant (DEB77-0341) to H. R. Pulliam.

Received 30 August 1979; accepted 11 March 1980.

1. Brown, J. L. *Am. Zool.* **14**, 51-62 (1974).
2. Cody, M. L. *Theor. Popul. Biol.* **4**, 142-158 (1971).
3. Krebs, J. R. *Behaviour* **51**, 99-134 (1974).
4. Treisman, M. *Anim. Behav.* **23**, 779-800 (1975).
5. Pulliam, H. R. *J. theor. Biol.* **38**, 419-422 (1973).
6. Siegfried, W. R. & Underhill, L. R. *Anim. Behav.* **23**, 504-508 (1975).
7. Caraco, T. *Ecology* **60**, 618-627 (1979).
8. Caraco, T. *Ecology* **60**, 611-617 (1979).
9. Pulliam, H. R. in *Perspectives in Ethology* (eds Klopfer, P. H. & Bateson, P. G.) 311-332 (Plenum, New York, 1976).
10. Hamilton, W. D. *J. theor. Biol.* **31**, 295-311 (1971).
11. Kenward, R. E. *J. Anim. Ecol.* **47**, 449-460 (1978).

Anti-micrococcus antibodies recognize an antigenic marker of confluent mouse lymphoid cell lines

J. Grooten, P. De Baetselier, E. Vercauteren & R. Hamers

Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Paardenstraat 65, 1640 St-Genesius-Rode, Belgium

In addition to the production of autoantibodies¹ and rheumatoid factors², other quite unique features accompany the immune response of various species to spontaneous infections or immunizations with cocci (streptococcus, pneumococcus, meningococcus, micrococcus). The immunoglobulin molecules produced are restricted in heterogeneity not only for antibodies raised against the bacteria³, but also for those raised against other antigens to which the animal is simultaneously immunized^{4,5}. In the case of micrococcus and streptococcus vaccinations where this has been investigated, dormant immunoglobulin (Ig) genes are activated and appear as new allotypes in the sera^{6,7}. Serum titres against maternal Ig light-chain allotypes increase considerably after immunization with micrococcus (W. van der Loo, personal communication). Such a pleiotropic effect suggests that bacterial vaccination affects the immune response at a very fundamental level. It was suggested that this aberrant behaviour of the immune response was driven by antibodies which cross-react with glycoprotein or glycolipid membrane components of the lymphoid cell population⁸. This was supported by the finding that purified anti-micrococcus antibodies, both *in vivo* and *in vitro*, affect the response of immunocompetent cells⁹. We now report that mouse anti-micrococcus antibodies recognize a membrane marker of mouse lymphoid cell lines, and that this marker is only exhibited after transition from non-confluent to confluent culture conditions.

Rabbit and mouse antibodies raised against micrococcus and streptococcus bind or agglutinate a variety of mammalian cells¹, including a small number of autologous lymphoid cells obtained from the immunized animal (R. Hamers, personal communication). Three cloned lymphoid cell lines were selected for further investigation: WEHI 7.1, which is a BALB/c T lymphoma¹⁰, the BALB/c myeloma Sp 2/0-Ag14, a non-producing variant of the Sp 2/HL-Ag14 line¹¹, and the BALB/c lymphoma S49 1/10 (ref. 12).

Binding of anti-micrococcus antibodies produced in BALB/c, CBA/Ca and C57BL mice was analysed with a fluorescence activated cell sorter (FACS) as a function of the culture condition and of the three cell lines. Fluorescein-labelled goat anti-mouse antibody was used as the fluorescent probe. After seeding, the cultures evolve from non-confluence with exponentially growing cells, to confluence and post-confluence conditions, in which the cells ultimately stop dividing.

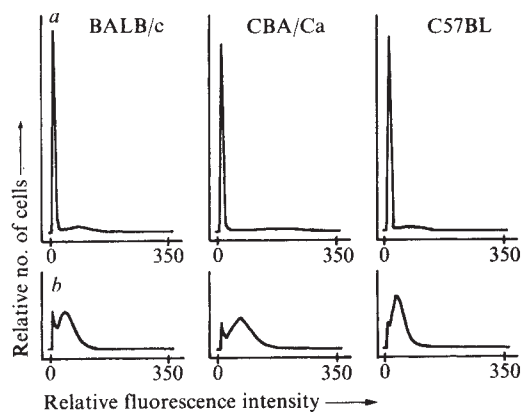


Fig. 1 Fluorescence histograms of WEHI 7.1 cells (cultured in Petri dishes) stained with anti-micrococcus antisera. The experimental protocol was as follows: 0.1 ml cells (5×10^7 cells ml⁻¹) in Dulbecco's balanced salt solution with 0.02% Na₂S₂O₈ (DBSS) were incubated with 0.010 ml antiserum (optimal concentration) at 4°C for 30 min followed by two washes with DBSS. After resuspension in 0.1 ml DBSS, 0.01 ml of an optimal dilution of fluorescein-labelled goat anti-mouse IgG (Nordic Immunological Laboratory) was added and the cells were incubated for 20 min at 4°C. After two washes with DBSS the cells were fixed overnight with formaldehyde (2% formaldehyde in DBSS) and analysed on the FACS II (Becton Dickinson Electronics Laboratory). A scatter window was set to eliminate dead cells and cell debris. The anti-micrococcus antisera were obtained with the following immunisation protocol: after one initial, intravenous injection of 0.2 ml (4 mg ml⁻¹ in saline) micrococcus suspension, the mice were injected 12 times intraperitoneally with the same amount over a period of 4 weeks. The animals were then given a rest period of 3 weeks, followed by a second course of nine intraperitoneal injections over 3 weeks. The sera were taken 2 weeks after the last injection. Less intense immunization protocols resulted in lower antibody titres. *a*, Early confluent culture conditions (12.5% stained cells); *b*, late confluent culture conditions (72.0% stained cells).

During the exponential growth phase no cells exhibited the membrane antigen detected by the antisera. When the cell density reached confluence, some cells began to express the marker, which in the final stage of confluence appeared on almost the entire population (Fig. 1). This phenomenon was observed on all three cell lines. Confluence cells of the Sp 2/0-Ag14 line were maintained at 37°C after staining to study capping. In these conditions, shedding of the fluorescein-labelled antibody was complete within 1 h as measured by the FACS. Control experiments with preimmune sera show only background fluorescence. An occasional preimmune serum,

however, will exhibit a weak binding on confluent cells, probably due to spontaneous anti-bacterial antibodies present in the serum of these conventionally maintained mice.

The marker appears as soon as confluence is reached and does not seem to depend on nutrient depletion, as can be shown in experiments in round-bottom tubes, where confluence is reached much earlier and at much lower cell densities than in Petri dishes. We will designate this marker as the confluence antigen (Cag).

The apparent synchronization between the appearance of the Cag marker and the transition from non-confluent to confluent conditions suggests that only the non-dividing or slowly dividing cells express the marker. To check this, the following experiment was carried out. ³H-Thymidine was added to cells at different stages of the culture and the ratio of ³H-thymidine uptake of fluorescent and non-fluorescent cells was measured after separation with the FACS. It follows from these experiments that the DNA synthesis, as measured by thymidine uptake, is much lower in the fluorescent cells (Cag⁺) than in the non-fluorescent cells (Cag⁻) obtained from the same culture (Table 1).

We conclude that the Cag marker appears on a cell population which showed reduced DNA synthesis in the 6 h before separation. From the serology of the reaction, some clues are obtained as to the nature of Cag. Adsorption of the antibodies on micrococcus lysodeikticus results in a decrease of the binding. Conversely, antibodies purified on a micrococcus cell-wall adsorbent¹³ and eluted with 0.2 M acetic acid were capable of staining the confluent cells, indicating that it is actually anti-micrococcus antibodies which bind to the Cag marker.

The confluence antigen is probably complex and several specificities and cross-reacting specificities could be involved. Antibodies from the different inbred strains do not stain the cells with the same intensities. Moreover, the binding inhibition patterns obtained using a panel of monosaccharides are qualitatively different for the three antisera (Table 2).

These results suggest that the Cag marker which is exhibited when the cell reaches confluence is a complex carbohydrate structure which comprises several different sugars. Further analysis to elucidate the carbohydrate composition of the Cag is now under way using hybridoma-produced anti-micrococcus antibodies.

Table 1 DNA synthesis of anti-micrococcus antibody-binding and non-binding cells

Culture stage	% Cells binding antibody	³ H-Thymidine incorporation (c.p.m. per 4×10^5 cells)			³ H-Thymidine uptake non-binding cells/binding cells
		Total cells	Binding cells	Non-binding cells	
Early confluent	5%	118,940	14,460	122,640	8.5
Late confluent	70%	6,669	795	19,325	24.3

WEHI 7.1 cells, cultured in Petri dishes, were tested for the rate of DNA synthesis at the beginning of confluence (early confluence) and 2 days later, when confluence was generalized (late confluence). A 6-h ³H-thymidine pulse was given to the cells followed by washing away the free ³H-thymidine and staining the cells as described in Fig. 1 legend. A fixed number (4×10^5) of fluorescent, non-fluorescent and total cells were separated on the FACS II and the ³H-thymidine incorporation of the three cell populations was estimated in a scintillation counter.

In conclusion, it seems that mouse anti-micrococcus antibodies recognize a membrane marker of murine lymphoid cell lines. This marker, which we call the confluence antigen (Cag), is only exhibited after transition from non-confluent to confluent culture conditions, and its expression is correlated with a slowing down or cessation of DNA synthesis.

Antigenic variation or heterogeneous expression of membrane antigens is not an isolated phenomenon in mammalian cells, and several authors¹⁴⁻¹⁶ have described the transient expression of blood group substances in relation to the mitotic cycle. Their observations, like ours, affect membrane glycoproteins or glycolipids and must be attributed to cell cycle-associated changes in the activity of the cellular glycosylating enzymes or their substrates.

Table 2 Inhibition of antibody binding by monosaccharides

Inhibitor	Anti-micrococcus antibody source		
	BALB/c	CBA/Ca	C57BL
None	100	100	100
D(+) glucose	67	76	100
L(-) glucose	55	95	100
α -D(+) fucose	84	95	100
α -L(-) fucose	61	91	100
D-mannose	71	68	67
D-galactose	67	81	67
N-Acetylglucosamine	60	100	53

Confluent stage WEHI 7.1 cells, showing 40% antibody binding, were suspended in DBSS (5×10^7 cells ml⁻¹) containing 0.14 M inhibitory monosaccharide. The suspension was incubated with antiserum (10%) for 30 min at 4°C, washed with DBSS and stained with fluorescein-labelled goat anti-mouse antibody as described in Fig. 1 legend. The cells were analysed on the FACS II and the decrease in fluorescence intensity estimated. Results given are values for relative fluorescence intensity at peak maximum. The position of the peak maximum is taken as 100 for the uninhibited control. The actual values for the three antisera are: BALB/c = 70, CBA/Ca = 92 and C57BL = 35 (see also Fig. 1).

The observed binding demonstrates the possibility of obtaining antibodies directed against membrane markers of neoplasms or other cells by immunization with a bacterial antigen. This is true even in the syngeneic system in which BALB/c antibodies react with cell lines of BALB/c origin. This could explain some of the suppression or enhancement effects of bacterial vaccination on tumour development^{17,18}. It is more difficult to determine the relevance of our findings to the peculiar behaviour of the immune system to micrococcus vaccination and to the observed effect of anti-micrococcal antibodies on *in vitro* systems. In staining normal lymphoid cells from BALB/c, CBA and C57BL mice, considerable variation was encountered between individual animals and we do not understand the reason for this variability.

This work was supported by a grant from the ASLK Kankerfonds and by the FGWO grant 30035.76. We thank Dr Kohler for a gift of the hybridoma cell lines, Dr Luckenbach for the WEHI 7.1 line, and Dr Wikler for the micrococcus cell-wall absorbent.

Received 22 October 1979; accepted 18 March 1980.

- Blue, W. T. & Lange, C. F. *Immunochimistry* **13**, 295-298 (1976).
- Hamers, R., Hamers-Casterman, C., van der Loo, W., Strosberg, A. D. & De Baetselier, P. *Z. Immunforsch. exp. Ther.* **149**, 187-192 (1975).
- Braun, D. G. & Jaton, J.-C. *Curr. Topics Microbiol. Immun.* **66**, 29-76 (1974).
- Montgomery, P. C. & Pincus, J. H. *J. Immun.* **111**, 42-51 (1973).
- De Baetselier, P., Hamers-Casterman, C., van der Loo, W. & Hamers, R. *Immunology* **33**, 275-284 (1977).
- van der Loo, W., De Baetselier, P., Hamers-Casterman, C. & Hamers, R. *Eur. J. Biochem.* **7**, 15-22 (1977).
- Mage, R. G., Rejnek, J., Glendowlyn, O., Young-Cooper, G. O. & Alexander, C. *Eur. J. Immun.* **7**, 460-468 (1977).
- De Baetselier, P., Hooghe, R., Grooten, J., Hamers-Casterman, C. & Hamers, R. *Proc. EORTC Meeting on Tumor Immunology: Clinical Tumor Immunology* (eds Wybran, J. & Staquet, M.) 253 (Pergamon, Oxford, 1976).
- De Baetselier, P., Grooten, J., Van De Winkel, M. & Hamers, R. *Archs intern. Physiol. Biochim.* **85**, 161-163 (1977).
- Stocker, J. W., Marchalonis, J. J. & Harris, A. W. *J. exp. Med.* **139**, 785-790 (1974).
- Shulman, M., Wilde, C. D. & Kohler, G. *Nature* **276**, 269-270 (1978).
- Horibata, K. & Harris, A. W. *Exp. Cell Res.* **60**, 61-77 (1970).
- Wikler, M. Z. *Immun. Forsch.* **149**, 193-200 (1975).
- Franks, D. & Dawson, A. *Exp. Cell Res.* **42**, 543-561 (1966).
- Kuhns, W. J. & Branson, S. *Nature* **219**, 938-939 (1968).
- Thomas, D. B. *Nature* **233**, 317-321 (1971).
- Collins, J. L. & Wust, C. J. *Cancer Res.* **34**, 932-937 (1974).
- Verloes, R., Atassi, G., Dumont, P. & Kanarek, L. *Br. J. Cancer* **38**, 599-605 (1978).

A monoclonal antibody specific for diploid epithelial cells in *Drosophila*

Danny L. Brower, R. J. Smith & Michael Wilcox

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Results from various experiments suggest that the cell surface has an important role in development¹. However, there is relatively little information on the specific surface molecules involved in developmental processes. In an effort to characterize cell-surface components that may be involved in *Drosophila* development, we have been making monoclonal antibodies against *D. melanogaster* imaginal disks². The holometabolous insects are unusual in that scattered among the larval tissues are groups of undifferentiated imaginal cells which, during metamorphosis, will form most of the adult insect^{3,4}. The imaginal disks, which we use as an immunogen, are hollow sacs of cells; each disk will form a specific part of the adult cuticle. Other imaginal cells are found as nests or rings in various larval organs. We describe here results indicating that one of the clones we have isolated, DA.1B6, makes an antibody against an antigen which, in larvae, is generally restricted to the undifferentiated sheets of imaginal epithelial cells. This and other results indicate that the antigen is specific for the diploid epithelia in *Drosophila*.

For the production of clone DA.1B6, a BALB/c mouse was immunized by injecting it intraperitoneally, on days 0, 5 and 12, with 200-300 sonicated imaginal disks in 0.1 ml *Drosophila* Ringer's solution (for all immunizations, and subsequent antibody binding experiments, tissues from the *D. melanogaster* Barton wild-type strain were used). On day 18 the mouse received a sonicate of 300 disks intravenously and, 2 days later, its spleen was removed. Spleen cells were fused with cells of the P3/NSI mouse myeloma line⁵ using polyethylene glycol, as described in ref. 6. When large colonies of hybrid cells had grown, the culture supernatants were screened for the presence of antibodies which would bind to intact imaginal disks, using indirect immunofluorescence (see legend to Fig. 1). After its identification as an antibody-secreting line, DA.1B6 was cloned twice, serially, using the soft agar technique⁷.

Table 1 catalogues the binding specificity of the antibody produced by DA.1B6. In late third instar larvae, the antibody binds to all of the imaginal disks (Fig. 1b), but it does not bind to all the cells of the disk. As shown in the section in Fig. 1c, the antibody binds to the epithelial cells of the wing disk, but not to the ad epithelial cells, which are believed to be precursors of at least some of the adult muscles^{8,9}. Binding is also observed to the imaginal rings in the salivary gland and gut, but there is no detectable binding to the large larval cells, either in these organs or elsewhere (Fig. 1d). In the adult, the antibody binds to the majority of the tissues which derive from these imaginal cells during metamorphosis.

The pattern of antibody binding to the larval and adult gonads is noteworthy. The ovaries of late third instar larvae show their strongest antigenic activity in a 'cap' across one end, with additional activity in a band further down the ovary (Fig. 1e). The lack of clear organization in the larval ovary makes this observation difficult to interpret, but the pattern of binding of the antibody to the adult ovary makes it likely that the larval binding is to precursors of the adult epithelia. In the adult organ, the antibody binds to the cells of the follicular epithelium surrounding the germarium and the adjacent anterior ovarioles (Fig. 1f). Small groups of cells at either end

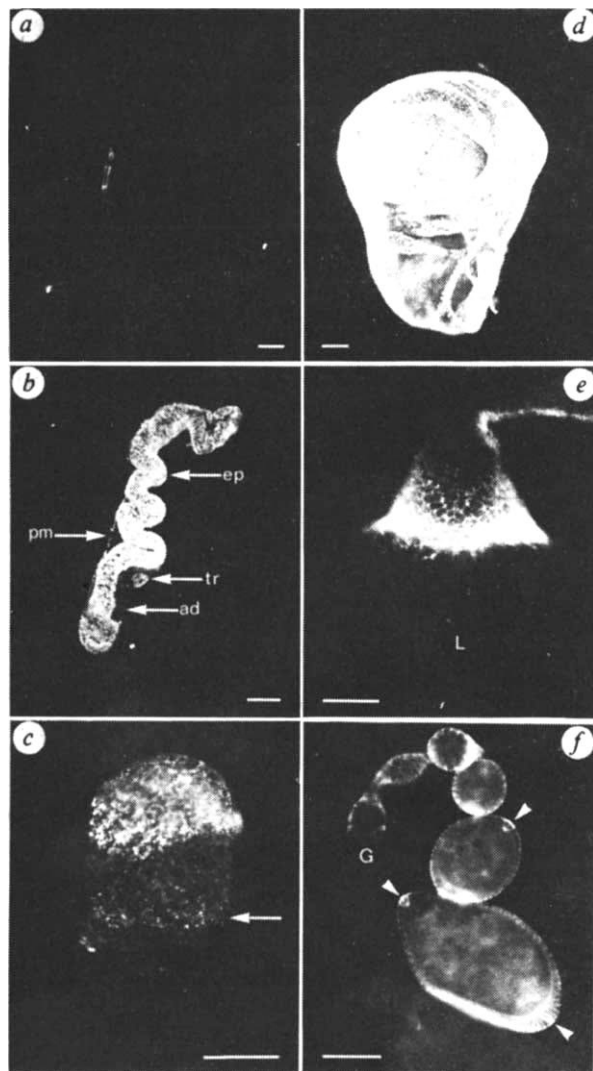


Fig. 1 Fluorescence micrographs of tissues from late third instar larvae (except *f*, which is from an adult ovary) stained with antibody from clone DA.1B6. Isolated tissues were incubated directly in cell culture supernatants, or in partially purified antibody diluted in RPMI medium (Gibco), for 45 min (30 min for sectioned material) at 37 °C (similar results were obtained at 23 °C). After three washes in cold medium, the tissues were incubated in fluorescein-conjugated rabbit anti-mouse IgG (Miles), diluted in RPMI medium, for 30 min at 37 °C (15 min for sections). After three more washes, the material was mounted and viewed with a Zeiss fluorescence microscope, using transillumination. *a*, Control wing disk incubated in RPMI medium without DA.1B6. *b*, Wing disk incubated with DA.1B6 antibody. *c*, Wing disk fixed with formaldehyde and frozen-sectioned before treatment with antibody. DA.1B6 binds to the cells from the columnar epithelium (ep) and the peripodial membrane (pm, which is in fact a squamous epithelium). The antibody also labels the matrix cells surrounding the tracheae (tr), but fails to bind to the adjacent adipocytes (ad). *d*, Salivary gland; the antibody binds to the ring of imaginal cells but not to the polyploid cells of the functioning larval gland (L). *e*, Ovary; binding is confined largely to a cap across one end of the organ, although an additional faint band of fluorescence (arrowed) is visible. *f*, Anterior end of an ovariole from an adult ovary. The follicle cells fluoresce brightly but there is no binding to the germ cells of the germarium (G). There is an exceptionally bright patch of cells at either end of each follicle (arrowheads). Follicles in later stages of oögenesis show relatively little fluorescence (bottom of figure). Scale bars, 50 µm.

of each follicle show especially bright fluorescence. There is no detectable binding to the germ cells in the germarium or to the developing nurse cells and oocytes. In the posterior part of each ovariole, where the follicle cells become polyploid and take an active part in vitellogenesis¹⁰⁻¹², the antibody fluorescence is generally much less intense than in the young follicles.

The bulk of the larval testis is antigen negative. There are, however, some localized areas of fluorescence on the surface of the testis; the most conspicuous is a small bright patch of cells at one end of the organ. In the adult gonad, the germ cells are again negative, but there is a bright fluorescent 'spot' at the distal end of the testis. These results suggest that, in both larvae and adults, there is binding to the apical cells of the germinal centre of the testis¹³.

Our preliminary study of embryos demonstrates that, early in development, the antigen recognized by DA.1B6 antibody is not restricted to imaginal precursor cells. Although we have not been able to detect any antibody binding to sections of embryos at the cellular blastoderm stage, by the end of germ band shortening (stage 12 of Bownes¹⁴) binding is evident to virtually the entire external epithelium and to many of the internal epithelia. By hatching, the antigen is disappearing from the cells that are differentiating into larval organs such as the salivary glands and gut, finally giving rise to the larval pattern of binding described above.

In summary, DA.1B6 antibody binds to the imaginal epithelial cells in third instar larvae, but not to the large cells of the larval organs. Many of the adult epithelia retain the binding capacity of their imaginal primordia. In early embryos, the antibody binds to most if not all of the epithelial cells, but, during later embryogenesis and early larval life, this binding activity disappears from those cells which form the larval organs.

Although the antibody generally seems to be specific for epithelial cells, it does not discriminate between germ layers, for there are examples of binding to cells from ectoderm (imaginal disks), endoderm (mid-gut) and mesoderm (follicle cells). The disappearance of the antigen from the larval

Table 1 Binding of antibody DA.1B6 to tissues of adults and third instar larvae

	Third instar larvae*	Adults
Epidermis†	—	
Imaginal disks: labial	+	+ ‡
dorsal prothoracic	+	
eye-antennal	+	
wing	+	
haltere	+	
leg	+	
genital	+	
Salivary glands	i	+
Gut†	i	+
Brain§	—	±
Thoracic muscles	—	—
Malpighian tubules	—	—
Fat body	—	—
Gonads	see text	

The binding assay is described in the legend to Fig. 1. — Little or no binding; +, binding to most or all of the cells; i, binding only to the imaginal rings of cells.

*Most of these tissues have also been examined in second instar larvae, always with identical results.

†We do not know if the imaginal histoblasts of the larval epidermis or mid-gut bind the antibody; the pupal mid-gut binds the antibody, but the activity is lost from the adult organ.

‡As judged by binding to thoracic and abdominal epithelium and to structures derived from the genital disk.

§A small region of the larval brain, sometimes seen to be associated with the eye-antennal disk, binds the antibody; binding to the adult brain is very heterogeneous.

epithelia may be related to the fact that these cells become polyploid during their differentiation⁴. This hypothesis is supported by the pattern of antibody binding to adult tissues. While most adult epithelia are recognized by the antibody, there is little or no binding to epithelia composed of polyploid cells (as indicated by Feulgen staining, our unpublished observations), such as the mid-gut. Also, in the adult ovary, there is a large reduction in the fluorescence of the follicle cells at about the time that they become polyploid during oogenesis¹⁰. The commitment to polyploidy by a cell entails a reduction in regulative capacity and a complete loss of proliferative potential. It seems quite possible, therefore, that the antigen is involved in these cellular processes.

Whatever the function of the antigen, the antibody can be useful as a developmental marker, particularly in organs such

as the gonads and brain, where the pattern of binding is heterogeneous. Moreover, the antibody may have biochemical applications, for example, in the purification of imaginal plasma membranes from whole larvae.

Without the unusual developmental history of the holometabolous insects, both the original identification and further characterization of DA.1B6 antibody would have been much more difficult. We believe that this and other unique features of *Drosophila* biology—for example, the elucidation of developmental compartments^{15,16} and the extensive knowledge of the fate and determination of imaginal disk cells¹⁷—make it the ideal organism for studies of this kind.

We would like to thank Bruce Wright for supplying P3/NSI cells, and numerous colleagues for suggestions and comments. D.L.B. is a fellow of the American Cancer Society.

Received 4 February; accepted 11 April 1980.

1. Karkinen-Jääskeläinen, M., Saxén, L. & Weiss, L. (eds) *Cell Interactions in Differentiation* (Academic, London, 1977).
2. Wilcox, M., Brower, D. & Smith, R. J. in *Development and Behaviour of D. melanogaster* (eds Siddiqi, O., Hall, L. & Babu, P.) (Plenum, New York, 1980).
3. Bodenstein, D. in *Biology of Drosophila* (ed. Demerec, M.) 275–337 (Hafner, New York, 1965).
4. Anderson, D. T. in *Developmental Systems: Insects* Vol. 1 (eds Counce, S. J. & Waddington, C. H.) 165–242 (Academic, London, 1972).
5. Köhler, G., Howe, S. G. & Milstein, C. *Eur. J. Immun.* **6**, 292–295 (1976).
6. Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).
7. Cotton, R. G. H., Secher, D. S. & Milstein, C. *Eur. J. Immun.* **3**, 135–140 (1973).
8. Poody, C. A. & Schneiderman, H. A. *With. Roux' Arch.* **166**, 1–44 (1970).

9. Reed, C. T., Murphy, D. & Fristrom, D. *With. Roux' Arch.* **178**, 285–302 (1975).
10. Mahowald, A. P., Caulton, J. H., Edwards, M. K. & Floyd, A. D. *Expl Cell Res.* **118**, 404–410 (1979).
11. King, R. C. *Ovarian Development in Drosophila melanogaster* (Academic, New York, 1970).
12. Mahowald, A. P. in *Developmental Systems: Insects* Vol. 1 (eds Counce, S. J. & Waddington, C. H.) 1–47 (Academic, London, 1972).
13. Hardy, R. W., Tokuyasu, K. T., Lindsley, D. L. & Garavito, M. J. *ultrastruct. Res.* **69**, 180–190 (1979).
14. Bownes, M. J. *Embryol. exp. Morph.* **33**, 709–801 (1975).
15. Garcia-Bellido, A., Ripoll, P. & Morata, G. *Dev Biol.* **48**, 132–147 (1976).
16. Crick, F. H. C. & Lawrence, P. A. *Science* **189**, 340–347 (1975).
17. Gehring, W. J. in *The Genetics and Biology of Drosophila* Vol. 2C (eds Ashburner, M. & Wright, T. R. F.) 511–554 (Academic, London, 1978).

K562 human leukaemic cells express fetal type (i) antigen on different glycoproteins from circulating erythrocytes

Minoru Fukuda

Biochemical Oncology, Fred Hutchinson Cancer Research Center, and Department of Pathobiology, School of Public Health, University of Washington, Seattle, Washington 98104

During the ontogenic change from fetal to adult human erythrocytes, as well as fetal haemoglobin being replaced by adult haemoglobin, the cell-surface antigen i is converted to I (ref. 1). Recently it has been shown that this antigenic change is the conversion of the linear repeating $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 3\text{Gal}$ structure to branched $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 3(\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 6)\text{Gal}$ structure^{2,4}. We have shown that cell-surface labelling followed by endo- β -galactosidase digestion can distinguish these two forms on the cell surface, and that band 3 and band 4.5 are the major carriers for these antigens on mature erythrocytes⁵. Human leukaemic cell line K562, originally isolated from a patient at blast crisis of chronic myelocytic leukaemia⁶, has recently been shown to synthesize glycophorin A⁷, and to be capable of synthesizing haemoglobin upon induction^{8,9}. I demonstrate here that K562 cells express the fetal type (i) antigen on distinctly different glycoproteins from those of erythrocytes, by the use of cell-surface labelling followed by endo- β -galactosidase digestion or followed by immunoprecipitation with specific antibodies.

Cell-surface glycoproteins were labelled using galactose oxidase/ $\text{NaB}^{[3}\text{H}]_4$ (ref. 10) or by periodate/ $\text{NaB}^{[3}\text{H}]_4$ (ref. 11). Labelled cells were then digested by endo- β -galactosidase¹² and the released oligosaccharides and cell pellet were separated⁵. As shown in Fig. 1, oligosaccharides of various sizes were produced from adult erythrocytes while oligosaccharides of smallest molecular weight were produced from the cord erythrocytes. The former pattern was derived from the branched polyactosaminyl structure with I-type, while the latter was derived from non-branched polyactosaminyl structure with i-type, as demonstrated previously^{4,5}. Cell-surface labelled K562 cells showed the

oligosaccharide pattern essentially the same as fetal type (i) (Fig. 1c,d). The K562 cells labelled by $^{[3}\text{H}]$ -GlcN produced disaccharide, trisaccharide and tetrasaccharide with endo- β -galactosidase digestion (Fig. 1e). If we assume the specific radioactivities of glucosamine and sialic acid in oligosaccharides derived from $^{[3}\text{H}]$ -GlcN labelled cells are the same, the molar ratio of disaccharide to tri- plus tetrasaccharide is calculated to be 3.4:1.0, based on the radioactivities recovered from paper chromatography. This value was fairly close to that (3.2:1.0) obtained on band 3 from i variant adult⁴. These data, together with the results of immunofluorescence staining by anti-i and anti-I sera (data not shown), indicate that fetal (i) antigen with the linear polyactosaminyl structure is present on the cell surface.

Cell-surface glycoproteins were examined before and after endo- β -galactosidase digestion. As shown in Fig. 2, bands 3 and 4.5, which are major Ii antigenic carriers in mature erythrocytes⁵, seemed to be absent. Instead, two bands in the band 3 region and PAS 2 were slightly affected by endo- β -galactosidase digestion. However, a glycoprotein at the position of band 3, which was heavily labelled by periodate/ $\text{NaB}^{[3}\text{H}]_4$ or metabolic labelling, was scarcely affected by endo- β -galactosidase, suggesting that this glycoprotein, designated as Gp105 according to a previous report¹³, is different from the band 3 glycoprotein present on matured cells. The extent of radioactivity released by endo- β -galactosidase treatment also revealed a significant difference between K562 cells and matured erythrocytes. Galactose oxidase/ $\text{NaB}^{[3}\text{H}]_4$ labelled K562 cells released only 10% of incorporated radioactivity, whereas 40% was released from erythrocytes. On the other hand, 7% of incorporated radioactivity was released from periodate/ $\text{NaB}^{[3}\text{H}]_4$ labelled K562 cells, whereas 5% was released from erythrocytes. The results support the above conclusion that K562 cells express a minimum amount of glycoproteins carrying polyactosaminyl structure, particularly those labelled by galactose oxidase/ $\text{NaB}^{[3}\text{H}]_4$.

Cell-surface labelling followed by immunoprecipitation was then carried out. As shown in Fig. 3, anti-band 3 antiserum precipitated almost no band from cell-surface labelled K562 cells, whereas anti-glycophorin antiserum precipitated PAS 2 from the same cell line. A small amount of Gp105 also

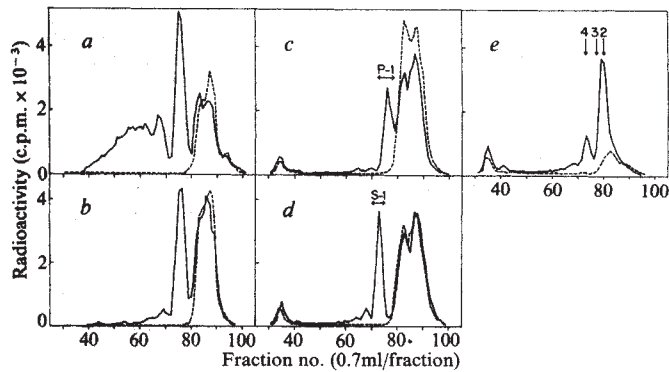


Fig. 1 Gel filtration of oligosaccharide released by endo- β -galactosidase from cell-surface labelled or metabolically labelled cells. Sephadex G-50 (1.0 $\times$ 94 cm) was equilibrated and eluted with 0.2 M NaCl. Aliquots (10 μ l for a, b; 100 μ l, c, d; 300 μ l, e) of each fraction were taken for measuring radioactivity. K562 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum. After washing with 0.01 M sodium phosphate buffer, pH 7.4 containing 0.14 M NaCl, 1 mM CaCl_2 and 1 mM MgCl_2 (PBS), the cells were labelled by galactose oxidase/ $\text{NaB}^{[3]\text{H}}_4$ (ref. 10) or periodate/ $\text{NaB}^{[3]\text{H}}_4$ (ref. 11). K562 cells were also metabolically labelled with $^{[3]\text{H}}$ -GlcN as described previously¹⁷. Cell-surface or metabolically labelled K562 cells were digested with endo- β -galactosidase as described previously⁵. Cell supernatant after incubation with endo- β -galactosidase (—) and cell supernatant after incubation without endo- β -galactosidase (-----). Oligosaccharides from galactose oxidase-labelled adult erythrocytes (a) or galactose oxidase-labelled umbilical cord erythrocytes (b) or galactose oxidase-labelled K562 cells (c) or periodate labelled K562 cells (d) or $^{[3]\text{H}}$ -GlcN metabolically labelled K562 cells (e) were applied. Oligosaccharides from periodate labelled cord erythrocytes showed a similar pattern to those from periodate labelled K562 cells⁵. The radioactive peaks in Fraction 80 to 100 are inorganic tritium salt (a-d) or $^{[3]\text{H}}$ -GlcN monosaccharide (e).

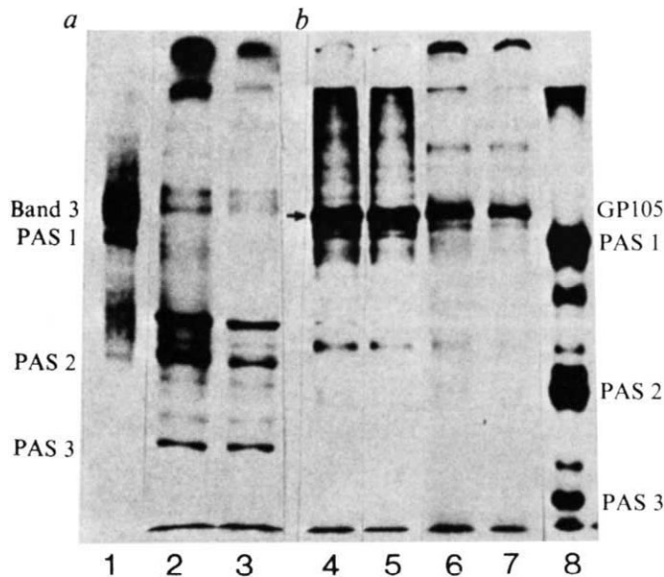


Fig. 2 Fluorograph of SDS-polyacrylamide gels of cell-surface or metabolically labelled cells. Cell pellets obtained as described in Fig. 1 legend were analysed by SDS-polyacrylamide gel electrophoresis¹⁸, using 8% acrylamide concentration in separation gel, and gels were treated for fluorography as described previously¹⁹. Lane 1, galactose oxidase-labelled adult erythrocytes; Lane 8, periodate-labelled adult erythrocytes; Lanes 2, 3, galactose oxidase labelled K562 cells; Lanes 4, 5, periodate-labelled K562 cells; Lanes 6, 7, $^{[3]\text{H}}$ -GlcN metabolically labelled K562 cells. Lanes 2, 4 and 6 are control cells incubated without endo- β -galactosidase and Lanes 3, 5 and 7 are cells incubated with endo- β -galactosidase. Electrophoresis of a and b were done on different occasions. The numerical designation for the major proteins of human erythrocyte is according to Steck²².

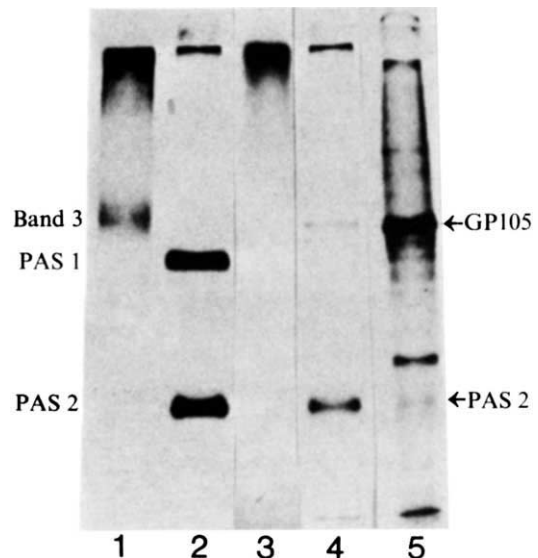


Fig. 3 Fluorograph of SDS-polyacrylamide gels of immunoprecipitates with anti-band 3 antiserum or with anti-glycophorin antiserum. Cell-surface labelled cells were lysed in 0.5% Triton X-100 in PBS containing 1 mM phenylmethylsulphonylfluoride and the extract obtained by centrifugation was treated with anti-band 3 antiserum²⁰ or anti-glycophorin antiserum (prepared as usual) followed by *Staphylococcus aureus*²¹. The precipitate thus obtained was analysed by SDS-polyacrylamide gel electrophoresis. Lane 1, galactose oxidase-labelled erythrocytes with anti-band 3 antiserum. Lane 2, periodate-labelled erythrocytes with anti-glycophorin antiserum. Each pre-immune serum showed negligible amounts of bands. Lane 3, periodate-labelled K562 cells with anti-band 3 antiserum; Lane 4, periodate-labelled K562 cells with anti-glycophorin antiserum. Lane 5, periodate-labelled K562 cells. The amount of membrane used in lanes 3 and 4 is about four times that of lane 5. Galactose oxidase or $^{[3]\text{H}}$ -GlcN labelled K562 cells with anti-band 3 antiserum showed negligible amounts of bands in the same conditions.

coprecipitated with anti-glycophorin antiserum. This was probably nonspecific precipitation as the ratio of Gp105 to PAS 2 was greatly reduced after immunoprecipitation (compare gel 4 and 5 of Fig. 3). Thus Gp105 is different from band 3 or glycophorin.

It is interesting that leukaemic cell line K562 expresses fetal (i) antigen as the elevated expression of i antigen has been reported in erythrocytes of leukaemic patients¹⁴. This finding may be also relevant to the production of fetal and embryonic haemoglobins by K562 cells upon induction with haemin⁹. The cell-surface glycoprotein profile of this cell line is shown here to be distinctly different from that of mature erythrocytes, although both shared glycophorin as described previously¹³. In addition, our recent study suggested that Gp105 was also present as a minor component on erythroblasts cultured *in vitro*¹⁵. It is possible that some of the unique cell-surface glycoproteins as well as its carbohydrate structure on K562 cells may provide surface markers specific to this tumour cell line or/and proerythroblasts of human cell lineage which are difficult to obtain *in situ*¹⁶.

I thank Dr Sen-itiroh Hakomori for his support, Dr Michiko N. Fukuda for endo- β -galactosidase, Dr Hansen for K562 cells, and Mr Edward Nudelman and Miss Carolyn Tozier for assistance in culturing cells. This work was supported by NIH grants CA19224 and CA23907.

Received 31 January; accepted 25 March 1980.

1. Marsh, W. L. *Br. J. Haemat.* **7**, 200-209 (1961).
2. Watanabe, K., Hakomori, S., Childs, R. A. & Feizi, T. *J. biol. Chem.* **254**, 3221-3228 (1979).
3. Feizi, T., Childs, R. A., Watanabe, K. & Hakomori, S. *J. exp. Med.* **149**, 975-980 (1979).
4. Fukuda, M., Fukuda, M. N. & Hakomori, S. *J. biol. Chem.* **254**, 3700-3703 (1979).

5. Fukuda, M. N., Fukuda, M. & Hakomori, S. *J. biol. Chem.* **254**, 5458–5465 (1979).
6. Lozzio, C. B. & Lozzio, B. B. *Blood* **45**, 321–334 (1975).
7. Jokinen, M., Gahmberg, C. G. & Andersson, L. C. *Nature* **279**, 604–607 (1979).
8. Andersson, L. C., Jokinen, M. & Gahmberg, C. G. *Nature* **278**, 364–365 (1979).
9. Rutherford, T. R., Clegg, J. B. & Weatherall, D. J. *Nature* **280**, 164–165 (1979).
10. Gahmberg, C. G. & Hakomori, S. *J. biol. Chem.* **248**, 4311–4317 (1973).
11. Gahmberg, C. G. & Andersson, L. C. *J. biol. Chem.* **252**, 5888–5894 (1977).
12. Fukuda, M. N. & Matsumura, G. *J. biol. Chem.* **251**, 6218–6225 (1976).
13. Gahmberg, C. G., Jokinen, M. & Andersson, L. C. *J. biol. Chem.* **254**, 7442–7448 (1979).
14. Giblett, F. R. & Crookston, M. C. *Nature* **201**, 1138–1139 (1964).
15. Fukuda, M., Fukuda, M. N., Papayannopoulou, Th. & Hakomori, S. *Proc. natn. Acad. Sci. U.S.A.* **77** (in the press).
16. Harrison, P. R. *Nature* **262**, 353–356 (1976).
17. Fukuda, M. & Hakomori, S. *J. biol. Chem.* **254**, 5442–5450 (1979).
18. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
19. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
20. Fukuda, M., Eshdat, Y., Tarone, G. & Marchesi, V. T. *J. biol. Chem.* **253**, 2419–2428 (1978).
21. Tonegawa, Y. & Hakomori, S. *Biochem. biophys. Res. Commun.* **76**, 9–17 (1977).
22. Steck, T. L. *J. Cell Biol.* **62**, 1–19 (1974).

5-Methoxypsoralen, an ingredient in several suntan preparations, has lethal, mutagenic and clastogenic properties

M. J. Ashwood-Smith*, G. A. Poulton†, M. Barker* & M. Mildenberger*

*Department of Biology and †Department of Chemistry, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2

Many furocoumarins found in several species of plant^{1,2} are potent photosensitizing agents known to cause lethal and mutagenic effects in a wide range of organisms, from viruses to man³. Their role in the aetiology of cancer is debatable^{3,4}, but work has focused on the PUVA (psoralen-UVA) treatment of psoriasis with 8-methoxypsoralen (8-MOP) and near UV radiation⁵⁻¹⁸. Bergapten (5-methoxypsoralen, 5-MOP) is a major constituent of oil of bergamot^{1,19}, and might be expected to have qualitatively similar photosensitizing properties to 8-MOP. Although 5-MOP is widely used as a stimulus to melanin deposition in several suntan preparations surprisingly little is known about its basic photobiology². We report here that 5-MOP has the expected properties of other biologically active furocoumarins. These properties include lethal and mutagenic photosensitization of bacteria, 'dark' induced frameshift mutagenesis in bacteria, and lethal and clastogenic effects on mammalian cells in tissue culture.

The use of 5-MOP in a number of suntan preparations widely available in several European countries has been questioned²⁰. It was suggested, in view of the evidence of the carcinogenicity of 8-MOP when combined with near UV light^{3-8,15,16}, that 5-MOP might have similar properties. There has been one limited study reporting lethal effects of 5-MOP on mouse tumour cells²¹, and a brief report on the production of chromosome damage in onion cells²². However, there is some information on skin photosensitization and some biochemical effects of 5-MOP, such as binding to DNA and RNA²³⁻²⁵: studies have shown 5-MOP to be less active than 8-MOP or psoralen but more active than angelicin (see Fig. 1).

5-MOP is not available commercially but is present in expressed oil of bergamot, the essential oil of *Citrus bergamia* to the extent of 0.30–0.36% (ref. 19). Nearly all samples of bergamot oil sold in the US have been steam distilled and contain no furocoumarins, as these compounds are considered undesirable in perfumery. Pure 5-MOP was isolated²⁶ from oil of bergamot (Sigma), an expressed oil containing furocoumarins, as no simple method of 5-MOP synthesis has

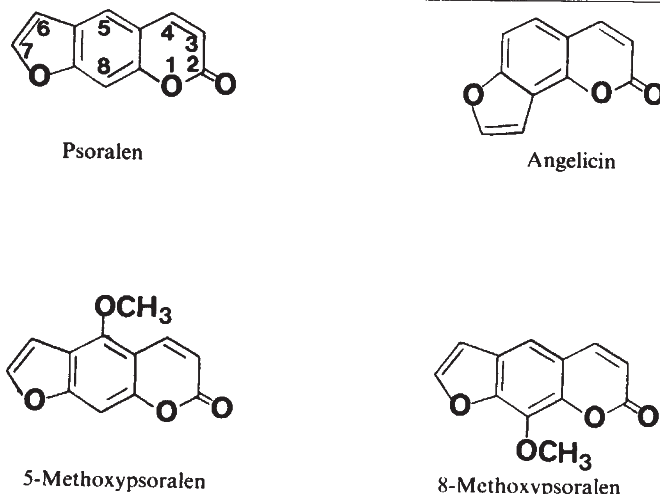


Fig. 1 Structural formulae of furocoumarins.

been reported. A series of photobiological studies on pure 5-MOP were compared with those of other furocoumarins.

Lethal photosensitization of *Escherichia coli* by several furocoumarins is shown in Fig. 2. Neither UV radiation alone nor any one of the test compounds was active unless combined. These results are similar to those generally seen with furocoumarins²⁷⁻²⁹. The effectiveness of 5-MOP in producing lethal damage relative to the most active compound, psoralen and the least active, angelicin, was essentially predictable from the early studies on photoreactivity with nucleic acids, skin photosensitization and lethal effects on tumour cells^{21,24}.

The mutagenic activity of 5-MOP, in the presence of near UV light, relative to three other furocoumarins is shown in Fig. 3. Base-pair substitutions were assayed in *E. coli* WP2 try⁻. As with the studies on lethality the order of effectiveness ranked psoralen as the most active compound and angelicin as

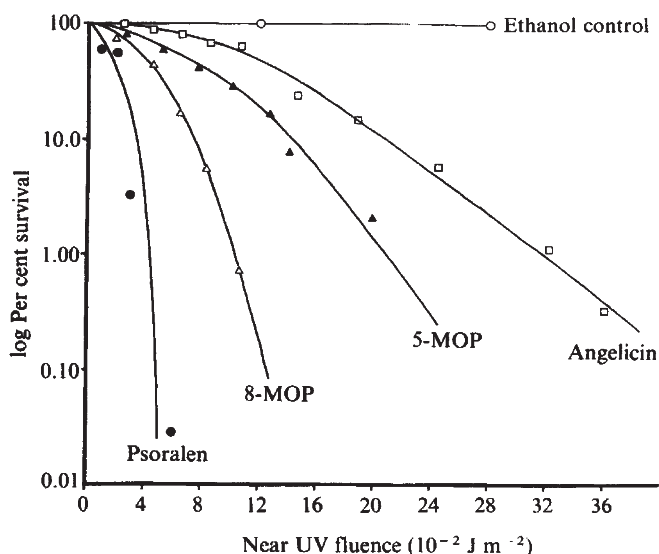


Fig. 2 Lethal photosensitization of *E. coli* with various furocoumarins (40 µg ml⁻¹ each). *E. coli* WP2 try⁻ was grown in nutrient broth at 22 °C and when the cells were at a density of ~5 × 10⁸ cells per ml (logarithmic growth stage) they were filtered and resuspended in 0.07 M phosphate buffer at pH 7.2 before irradiation which was 5 min after the addition of the furocoumarins. Near UV (320–380 nm) was produced by two parallel GEC black lamps (F20T12.BLB) emitting 13.4 J m⁻² as measured by chemical actinometry. Cells were plated on nutrient agar and colonies counted after 24 h of incubation at 37 °C.

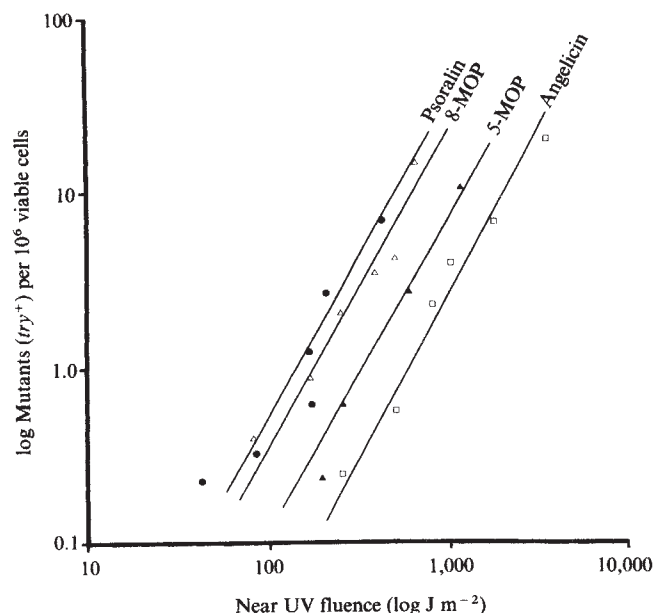


Fig. 3 Mutagenesis in *E. coli* induced by furocoumarins ($40 \mu\text{g ml}^{-1}$) and near UV light. *E. coli* WP2 *try*⁻ was grown as described in Fig. 2 legend. Handling and irradiation conditions were identical. Mutant (*try*⁺) cells were detected on minimal plates 48 h after incubation²⁷.

the least active. The slope of the log-log plot suggests a two-hit mechanism (R. C. von Borstel, personal communication) of mutagenesis, which is basically similar for all the furocoumarins.

The inability of angelicin to form DNA interstrand cross-links has been reported²⁸⁻³¹, and the slope of the angelicin curve is not easily explained. Interstrand DNA cross-links are implicated in death caused by photosensitizing furocoumarins^{32,33}; their role in mutagenesis has only recently been studied^{28,29}. The mutation system used in this experiment is well understood and is related to either true suppression of an ochre stop codon in the tryptophan synthetase pathway or reversion. Some of the original observations of mutagenesis with 8-MOP in *E. coli* were performed with this same test system²⁷.

Several reports have indicated that 8-MOP acts as a weak frameshift mutagen in bacteria, *per se*, in the absence of light^{34,35}. Recently M.J.A.-S.³⁶ reported that angelicin and psoralen were frameshift mutagens in *E. coli* although surprisingly, 4,5',8-trimethylpsoralen was inactive. Trimethylpsoralen is one of the most active photosensitizing compounds, however. Experiments with *E. coli lac*⁻ (strain ND160) plus 5-MOP indicate that it is active as a frameshift mutagen. The rank order (relative to a standard ethanol control which gave 40 ± 23 ($\pm$ s.d.) *lac*⁺ mutants per 1×10^8 viable cells and 346 ± 49 ($\pm$ s.d.) *lac*⁺ mutants with psoralen) was as follows: control, 1.0; 5-MOP, 2.4; 8-MOP, 5.1; angelicin, 5.9; and psoralen, 8.6. All compounds were tested at $40 \mu\text{g ml}^{-1}$.

control, 1.0; 5-MOP, 2.4; 8-MOP, 5.1; angelicin, 5.9; and psoralen, 8.6. All compounds were tested at $40 \mu\text{g ml}^{-1}$. The lethal photosensitizing effect on Chinese hamster cells in tissue culture is illustrated in Fig. 4a. The results with psoralen are remarkably similar to those with 8-MOP and Chinese hamster cells³⁷. Again, the three furocoumarins tested had the same ranked order of effectiveness in lethal photosensitization with mammalian cells as they had with bacterial cells.

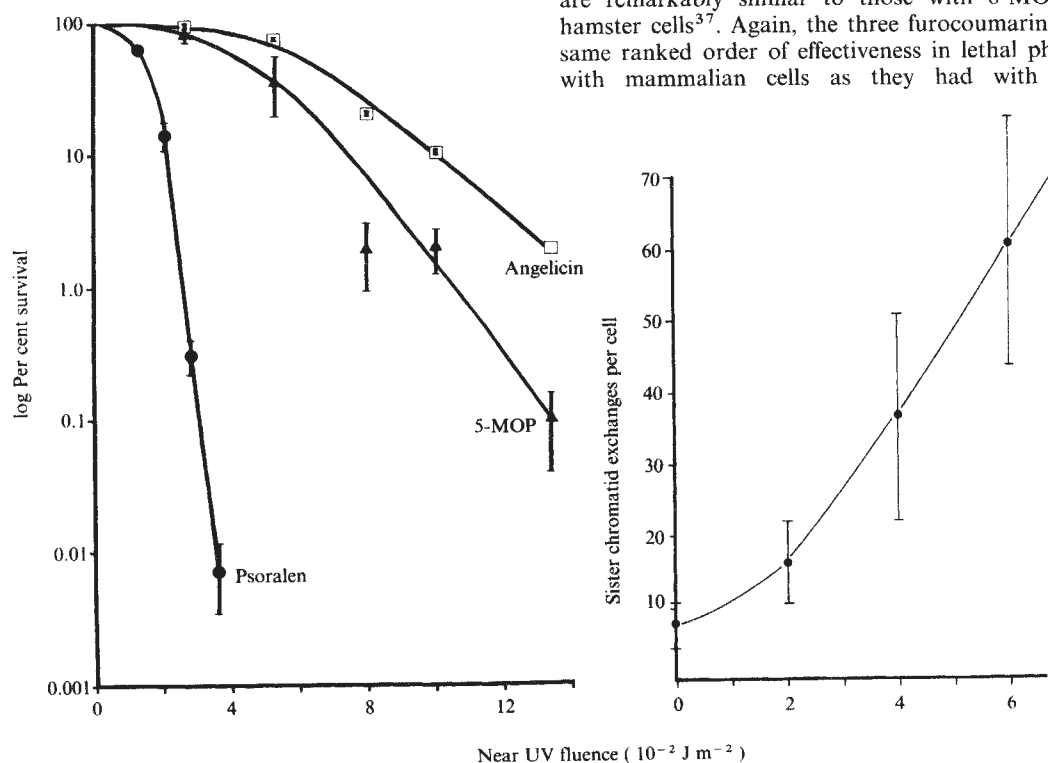


Fig. 4 *a*, Lethal photosensitization of Chinese hamster cells in tissue culture with furocoumarins ($40 \mu\text{g ml}^{-1}$). Chinese hamster cells (CHO) were grown in McCoy's medium supplemented with 15% fetal calf serum. The handling and irradiation conditions have been described³⁸. Cells, attached to the surface of plastic flasks, were washed with tissue culture medium minus serum. Furocoumarins dissolved in 95% ethanol were added 10 min before irradiation. When the irradiation was completed fresh medium containing serum was added and the cells incubated for 10 days at 37°C before colony counts were made. Results are expressed as per cent survival $\pm$ s.d. *b*, Sister chromatid exchanges in Chinese hamster cells induced by 5-MOP and near UV. Chinese hamster cells were grown and irradiated as in *a*. Standard methods for the detection of SCEs were used by growing cells for 30 h in the presence of bromodeoxyuridine after irradiation and using staining and fixation procedures essentially as described by Perry and Wolff⁴⁵. A division delay after furocoumarin photosensitization has been observed³⁸ and therefore 30 h rather than 24 h (about two divisions for normal CHO cells) was chosen as the collection time. Error bars, $\pm$ s.d.

Chromosome damage in Chinese hamster cells treated with psoralen, or angelicin and near UV has been reported³⁸, and photosensitized cell death and mutations in surviving cells with 8-MOP has been demonstrated in several different mammalian systems^{6,7,37,39}, including diploid human skin fibroblasts in tissue culture⁴⁰. The formation of sister chromatid exchanges (SCEs) when Chinese hamster cells were irradiated with near UV in the presence of $40\text{ }\mu\text{g ml}^{-1}$ 5-MOP is illustrated in Fig. 4b. The dose-response curve is normal and the points chosen represent lethality values induced by the treatment within a range of 5–95% kill. 8-MOP yielded SCE values similar to those obtained with 5-MOP at equivalent cell survival. The formation of SCEs in human diploid skin cells after 8-MOP and UV irradiation has recently been reported⁴⁰ and lymphocytes from patients treated with 8-MOP showed increased SCEs when irradiated *in vitro*⁴¹. However, after PUVA therapy there has not generally been an observable increase in SCEs⁴⁰.

Interrupted irradiation experiments similar to those described for trimethylpsoralen with tissue culture cells⁴² and for bacteria with angelicin and psoralen³¹ suggested that 5-MOP forms DNA interstrand cross-links. The slope of the bacterial survival curve was only slightly changed when irradiation was continued after removal of free 5-MOP from cells irradiated with UV light to a point which produced a kill of 10%. In contrast, experiments with angelicin, which cannot form cross-links, have resulted in an abrupt cessation in the lethality induced by irradiation when angelicin was removed³¹. Preliminary experiments using alkaline sucrose density gradient analysis indicate the formation of DNA interstrand cross-links, albeit perhaps less efficiently than with psoralen or 8-MOP.

We conclude that 5-MOP produces similar photobiological responses to 8-MOP, a chemical clearly possessing mutagenic, clastogenic and carcinogenic properties in conjunction with near UV radiation.

The use of 5-MOP in suntan preparations constitutes, in our judgement, a definite and unnecessary hazard. The concentrations of 5-MOP present in the most widely used preparations vary from 12 to $50\text{ }\mu\text{g ml}^{-1}$. Our studies with 5-MOP were conducted with $40\text{ }\mu\text{g ml}^{-1}$. The possible advantages derived from increased pigmentation several days after the topical application of 5-MOP are the enhanced protection against the known biological effects of sunlight. However, the problem of the molecular and chromosomal damage likely to be produced in human skin before the formation of pigment seems to us to greatly outweigh any benefits. Individuals at risk also include those bathing in swimming pools containing 'washed-off' 5-MOP.

As we learn more about the distribution of heterozygotic genes that affect DNA repair⁴³ the proportion of individuals at risk may be found to be much higher than anticipated. Worthy of note in this context are the unfortunate experiments where treatment of *Xeroderma pigmentosum* patients with 8-MOP and near UV resulted in skin cancers⁴⁴. An epidemiological study of skin cancers and melanomas in which the role of these preparations is examined is clearly necessary.

We thank Mrs Carol Warby for technical assistance, Dr W. Steck for a gift of reference 5-MOP, Professor M. Wilkshire for help and information, Dr. Jean-Michel Turc and Professor Bryn Bridges for discussions. M.J.A.-S. and G.A.P. were supported by the Natural Sciences and Engineering Research Council of Canada.

Note added in proof: Studies begun in August 1978 at the Institut du Radium, Orsay, France, have shown that 5-MOP in combination with near UV is almost as potent a skin carcinogen in mice as 8-MOP (F. Zajdela, personal communication, April 1980).

- Mitchell, J. & Rook, A. in *Botanical Dermatology* (Greengrass, Vancouver, 1979).
- Scott, B. R., Pathak, M. A. & Mohn, G. R. *Mutat. Res.* **39**, 29–74 (1976).
- Urbach, F. J. *invest. Derm.* **32**, 373–378 (1959).
- Griffin, A. C. J. *invest. Derm.* **32**, 367–372 (1959).
- Forbes, P. D., Davies, R. E. & Urbach, F. *J. Cosmet. Toxicol.* **14**, 303–306 (1976).
- Burger, P. M. & Simons, J. W. I. M. *Bull. Cancer* **65**, 281–282, 3 (1978).
- Evans, D. I. & Morrow, K. J. *invest. Derm.* **72**, 35–41 (1979).
- Grube, D. D. *Proc. Am. Ass. Cancer Res.* **16**, 117 (1975).
- Epstein, J. H. & Fukuyama, K. J. *invest. Derm.* **54**, 350–351 (1970).
- Walter, J. F. & Voorhees, J. J. *Acta derm.-venereol. Stockh.* **53**, 469–472 (1973).
- Weber, G. *Br. J. Derm.* **90**, 317–323 (1974).
- Parrish, J. A., Fitzpatrick, T. B. & Tanenbaum, L. *New Engl. J. Med.* **291**, 1207–1211 (1974).
- Wolf, K., Fitzpatrick, T. B. & Parrish, J. A. *Arch. Derm.* **112**, 943–950 (1976).
- Meliski, T., Tanenbaum, L. & Parrish, J. A. *invest. Derm.* **68**, 328–335 (1977).
- Stern, R. S., Thibodeau, L. A., Kleinerman, R. A., Parrish, J. A. & Fitzpatrick, T. B. *New Engl. J. Med.* **300**, 809–813 (1979).
- Epstein, J. H. *New Engl. J. Med.* **300**, 852–853 (1979).
- Ashwood-Smith, M. J. & Grant, E. L. *Br. med. J.* **i**, 342 (1976).
- Strauss, G. H. & Albertini, R. J. *invest. Derm.* **73**, 211 (1979).
- Cieri, U. R. *J. Ass. off. analyt. Chem.* **52**, 719–728 (1969).
- Ashwood-Smith, M. J. *Br. med. J.* **ii**, 1144 (1979).
- Musajo, L., Visentini, P., Baccichetti, F. & Razzi, M. A. *Experientia* **23**, 335–336 (1967).
- Musajo, L. *Farmacol.* **10**, 2–7 (1955).
- Musajo, L. & Rodighiero, G. *Prog. Biochem. Pharmac.* **1**, 366–371 (1965).
- Rodighiero, G. *et al. Experientia* **25**, 479–481 (1969).
- Rodighiero, G. *et al. Biochim. biophys. Acta* **217**, 40–49 (1970).
- Steck, W. *Phytochemistry* **9**, 1145–1146 (1970).
- Igali, S., Bridges, B. A., Ashwood-Smith, M. J. & Scott, B. R. *Mutat. Res.* **9**, 289–295 (1970).
- Averbeck, D. P., Chandra, P. & Biswas, R. F. *Radiat. envir. Biophys.* **12**, 241–252 (1975).
- Grant, E. L., von Borstel, R. C. & Ashwood-Smith, M. J. *Envir. Mutat.* **1**, 55–63 (1979).
- Rodighiero, G. *et al. Biophysik* **8**, 1–8 (1971).
- Ashwood-Smith, M. J. & Grant, E. *Experientia* **33**, 384–386 (1977).
- Cole, R. S. *Biochim. biophys. Acta* **254**, 30–39 (1971).
- Cole, R. S. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1064–1068 (1973).
- Clarke, C. H. *Mutat. Res.* **28**, 123–125 (1975).
- Bridges, B. A. & Mottershead, R. P. *Mutat. Res.* **44**, 305–312 (1977).
- Ashwood-Smith, M. J. *Mutat. Res.* **58**, 23–27 (1978).
- Burger, P. M. & Simons, J. W. I. M. *Mutat. Res.* **60**, 381–389 (1979).
- Ashwood-Smith, M. J., Grant, E. L., Heddle, J. A. & Friedman, G. B. *Mutat. Res.* **43**, 377–385 (1977).
- Arlett, C. F., Heddle, J. A., Broughton, B. C. & Rogers, A. M. *Clin. exp. Derm.* (in the press).
- Burger, P. M. & Simons, J. W. I. M. *Mutat. Res.* **63**, 371–380 (1979).
- Mourelatos, D., Faed, M. J. W., Gould, P. W., Johnson, B. E. & Frain-Bell, W. *Br. J. Derm.* **97**, 649–654 (1977).
- Ben-Hur, E. & Elkind, M. M. *Biochim. biophys. Acta* **331**, 181–193 (1973).
- Paterson, M. C., Anderson, A. K., Smith, B. P. & Smith, P. J. *Cancer Res.* **39**, 3725–3734 (1979).
- Reed, B. *Acta Derm.-venereol. Stockh.* **56**, 315–318 (1976).
- Perry, P. & Wollf, S. *Nature* **251**, 156–158 (1974).

Selective disruption of displacement behaviour by lesions of the mesolimbic dopamine system

Trevor W. Robbins

Department of Experimental Psychology,
Downing Street, Cambridge CB2 3EB, UK

George F. Koob

A. V. Davis Center for Behavioural Neurobiology,
Salk Institute, San Diego, California 92138

In the wild, organisms generally allocate their time among many behavioural tendencies in response to both current and anticipated motivational requirements¹. However, activities that are apparently 'irrelevant' often intrude, either during conflict between these behavioural tendencies, or when a strong tendency is thwarted. These 'irrelevant' activities are called displacement behaviours and are widely documented in the ethological literature^{2,3}. We report here that an experimental analogue of displacement behaviour in the rat depends upon the integrity of the mesolimbic dopaminergic projection to the nucleus accumbens septi, olfactory tubercle and associated structures of the forebrain^{4,6}.

Hungry rats exposed to the periodic presentation of small pellets of food, with temporal delay, in an experimental chamber develop excessive drinking, which can be explained neither in terms of physiological deficit, nor as a 'superstitious' result of the adventitious pairing of drinking with the delivery

Table 1 Neurochemical effects of 6OH-DA lesions of NAS region

Mean dopamine levels (ng per mg protein) (expt. 1)				
Group	n	NAS/OT	Anterior striatum	Posterior striatum
Sham	6	13.56 ± 2.42	19.41 ± 3.95	52.00 ± 4.67
Lesion	7	2.67 ± 0.62†	6.93 ± 2.36*	53.08 ± 7.09
% Depletion		80	64	

Mean tyrosine hydroxylase activity (pm per mg L-dopa per h) (expts 2 and 3)				
Group	n	NAS	OT	Corpus striatum
Sham and unoperated	12	282.2 ± 63.7	366.2 ± 66.5	442.7 ± 81.4
Lesion	12	39.6 ± 8.6†	65.5 ± 16.3†	304.1 ± 34.8
% Depletion		86	82	31

8 µg of 6OH-DA base were infused bilaterally in 2 µl vehicle at a rate of 1 µl per min through a 30-gauge stainless steel cannula. Stereotaxic co-ordinates were; anterior to bregma +3.4; lateral ±1.7; ventral from dura -7.2 mm, using the angle of Pellegrino and Cushman¹⁸. Rats received pretreatment with pargyline (50 mg per kg), and (in experiments 2 and 3 only) desmethylimipramine (DMI) (15 mg per kg), 30 min prior to operation. DMI was used to protect noradrenaline (NA) from depletion by 6 OHDA¹⁹, although NA depletion is probably not critical for these behavioural results¹⁷. A histological control, and details of the dissection procedure have previously been published¹⁷. Anterior striatum was dissected from the coronal slice containing nucleus accumbens. Nucleus accumbens (NAS) and olfactory tubercle (OT) were pooled in experiment 1. Dopamine was assayed by a radio-enzymatic technique^{20,21}. Tyrosine hydroxylase activity was measured by a method modified from Hendry and Iversen²². Values given are means ± s.e.m.

**P* < 0.05.

†*P* < 0.01, Student's *t*-test comparing lesion with control group.

of food⁷. Indeed, the incidence of other behaviours such as aggression, gnawing and wheel-running in comparable situations, for a variety of species including man, has encouraged the concept of a general category of 'adjunctive' behaviour, which is probably related to the more familiar displacement activities^{1,7-9}. One possible explanation of adjunctive behaviour is that the motivational excitement or activation accompanying the delivery of a food pellet may outlast its consumption, and potentiate alternative activities evoked by available environmental stimuli¹⁰. These acts are performed vigorously until interruption by competing behaviour emitted in anticipation of the next food pellet¹⁰. The ancillary motivational effect of the rewarding food pellet has been compared to that evoked by electrical stimulation of the lateral hypothalamus^{11,12}, which can itself engender adjunctive drinking¹³. Destruction of the lateral hypothalamus produces a reduction in adjunctive drinking¹⁴, but this lesion also produces a profound syndrome of aphagia and adipsia¹⁵, thus placing the specificity of the other effect in doubt. Major elements of the lateral hypothalamic syndrome have been attributed to damage of the ascending nigrostriatal dopamine (DA) projection which courses through the lateral hypothalamus¹⁶. A parallel dopamine projection, termed the meso-limbic DA system, originates in the ventral tegmental area (VTA) and innervates forebrain limbic areas such as the nucleus accumbens septi (NAS), olfactory tubercle (OT), and frontal cortex (FC)⁴⁻⁶. Recently, we found that destruction of the DA terminals in the NAS and OT did not produce primary motivational deficits, but did alter the apparent time-sharing among eating, drinking and locomotor activity in certain situations¹⁷. We now show that similar lesions attenuate adjunctive drinking, but not drinking induced by water deprivation, this report being the first of this type of dissociation. We also provide data on drinking and activity compatible with the hypothesis that the lesion attenuates adjunctive drinking by reducing activation and thus disrupting time-sharing among different activities.

In all experiments described here, destruction of DA terminals in NAS and OT was effected by stereotaxic, bilateral injections of 6-hydroxydopamine (6-OHDA) into the region of the NAS (see legend to Table 1) in anaesthetized male albino Wistar rats weighing about 300 g. Control rats received injections of vehicle (0.2% ascorbic acid in 0.9% saline) as sham

lesions, or in some cases, no operation at all. There were no differences in biochemical or behavioural parameters between sham-lesioned and unoperated rats, and hence their data are combined. Treatment with 6-OHDA produced reductions in activity of tyrosine hydroxylase (an enzyme involved in the biosynthesis of DA and noradrenaline), as well as regional depletion of DA in NAS/OT and the anterior striatum (Table 1).

In experiment 1, adjunctive drinking was induced by a standard procedure in lesioned and control rats²³. Following reduction to 85% of free-feeding weight, rats were familiarized on postoperative days 10–11, to a chamber containing a dispenser which could deliver food-pellets to a tray, and a spout connected to a graduated burette filled with water. The spout was situated 9.1 cm from the floor and 7.2 cm to the left of the food-tray. For the next 10 days, a single 45 mg food pellet (Noyes) was delivered to the tray every 60 s of the 30 min session. This schedule of delivery resulted in the gradual development of excessive drinking in 7 of 8 control rats, measured either in terms of volume of water consumed (Fig. 1), or by the number of licks recorded by a drinkometer circuit. This drinking cannot be attributed to a 'dry mouth' produced by the food pellets, since similar rats consuming about 8 times as much food of comparable nature over an identical time-period were found previously¹⁷ to drink only about 60% of the volume of water that these rats were ingesting by session 10. Cumulative pen-recordings of the drinking showed that it occurred predominantly in the initial portions of the period following food-delivery, the temporal locus characteristic of adjunctive behaviour⁷. In contrast to controls, rats with 6-OHDA lesions exhibited significant attenuation of adjunctive drinking, whether measured in terms of water-intake or licks recorded (Fig. 1). Dual measures were used partly because of the possibility of reduced lap volume or drinking efficiency in rats with pharmacological blockade of DA receptors in NAS²⁵. Inspection of our data supports the possibility of small impairments in drinking efficiency in rats with 6-OHDA lesions, but the parallel reductions in licking and intake rule out inefficiency of licking as a simple explanation of the reduced adjunctive drinking.

Experiments 2 and 3 show that the reduction of adjunctive drinking also cannot be explained as a deficit in primary thirst, but may be correlated with reduction in motivational activation as measured by locomotor activity and changes in time-sharing among different behaviours. In experiment 2, different groups of rats with 6-OHDA lesions, and controls,

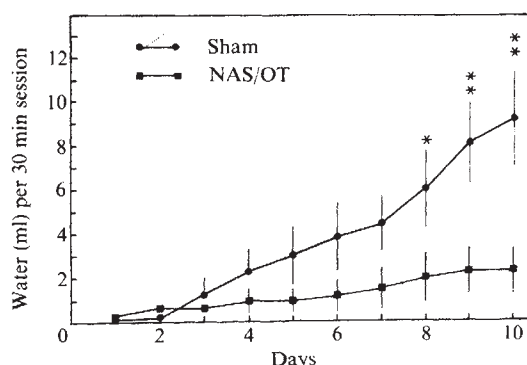


Fig. 1 Mean water intake in adjunctive drinking experiment. Asterisks indicate levels of statistical significance of difference between controls (*n* = 8) and rats with 6-OHDA lesions of NAS (*n* = 8). **P* < 0.05, ***P* < 0.01. Following analysis of variance with repeated measures, *F* values for days 8, 9, and 10 were respectively: 7.17, 15.45, and 22.43 (with 1 and 33 d.f., following Satterthwaite's approximation²⁴). Mean numbers of licks per 30 min of NAS and control groups for days 8, 9 and 10 were: NAS, 588.3, 642.1 and 681.9; control, 1418.9, 1835.3 and 2026.6, both respectively. These differences were significant beyond *P* < 0.01 for days 9 and 10, and beyond *P* < 0.05 for day 8.

Table 2 Behavioural results from experiment 2

Behavioural measure		n	Post-operative day			
			2	3	4	5
Locomotor activity (mean counts per 10 min)	NAS	12	108.5 ± 11.8	103.1 ± 9.2	101.6 ± 9.0	80.6 ± 8.8
	Control	12	194.7 ± 18.3	161.8 ± 15.2	147.9 ± 12.4	132.6 ± 14.4
Drinking (mean ml consumed per 30 min)	NAS	12	1.74 ± 1.05	11.02 ± 0.72	12.27 ± 0.36	12.23 ± 0.85
	Control	12	0.81 ± 0.36	13.74 ± 0.76	12.06 ± 0.76	12.17 ± 0.92

Rats were not deprived on postoperative day 2, but were deprived for 23 h on days 3, 4, and 5. The NAS group was significantly less active than controls on day 2 ($F=15.66$, d.f.=1,22, $P<0.001$), and on days 3, 4 and 5 ($F=17.45$, d.f.=1,22; $P<0.001$). There were no overall differences in mean water intake between the two groups, either on day 2 or days 3, 4 and 5 (both $F<1.00$, $P>0.05$).

were familiarized 2 days after surgery for 30 min to cages each containing a single photocell beam across the long axis¹⁷, situated 20 cm from one wall, to which were attached two burettes containing water. On 3 subsequent days, the rats were deprived of water for 23 h before testing. The rats with 6-OHDA lesions showed a highly significant reduction in locomotor activity but no significant lasting attenuation of water-intake compared with controls (see Table 2).

In experiment 3, we took the same deprived rats and studied their drinking patterns and activity individually over post-operative days 7–13, in an open field (50 × 35 cm) supplied with three burettes attached to one of the shorter walls and situated at 8 cm intervals, in three 15 min trials. Again, no significant effects of the 6-OHDA lesion on water-intake were found, although there were marked changes in the pattern of drinking exhibited. For example, the rats with lesions consumed significantly more water from their most preferred burette on the first two trials, as measured by the preference ratio (Table 3). In addition, observations on trials 1 and 3, made via closed circuit television, revealed that the rats with 6-OHDA lesions showed significantly longer bouts of drinking (Table 3). In addition, these rats made significantly fewer crossings among the four quadrants of the open-field (Table 3) thus confirming the hypoactivity found in the photocell cages.

In explaining these results it seems unlikely that the reduced adjunctive drinking is caused by a general sensory or motor debilitation given that the lesioned rats were all capable of locating and consuming the food delivered in experiment 1 and of drinking normal amounts in experiments 2 and 3. It remains possible that the reduction in adjunctive drinking results from an inability to move the small distance from the

food tray to the water tube, although inspection of individual data revealed that all the rats with lesions at least sampled the water-tube on every session. Alternatively, the hypoactivity as well as the attenuation of adjunctive drinking results from reduced motivational excitement in the rats with 6-OHDA lesions. This reduced activation leads to fewer interruptions of behaviour made dominant by deprivation¹⁷, or by previous training²⁶, thus explaining the initial 'focusing' of behaviour on a single burette and the changes in drinking pattern observed in experiment 3.

As expected, the 6-OHDA lesion produced a depletion of DA in NAS and OT. However, the demonstration of additional DA depletion in both the frontal cortex and the anterior striatum (Table 1 and ref. 17) poses the problem of defining the sufficient extent of DA depletion within the terminal areas critical for the observed behavioural effects. This problem is related to the definition of the mesolimbic DA system, itself. Recent anatomical evidence^{5,6} has shown considerable overlap in the innervation of the forebrain DA terminal regions by the DA cell bodies of the VTA and substantia nigra thus blurring the original distinction between the ascending nigrostriatal (A9) and mesolimbic (A10) projections. However, it is clear that 6-OHDA lesions of the portions of the DA terminals in the limbic forebrain produce very different effects from 6-OHDA lesions of the corpus striatum²⁷. Therefore, the behavioural effects we have found may help us to understand the function of a broadly-defined mesolimbic DA system encompassing, not only the NAS and OT, but also the frontal cortex and anterior striatum.

We believe these results to be important for several reasons. First, they provide an intriguing parallel to the severe deficits

Table 3 Behavioural results from experiment 3

Behavioural measure	Group	n	Post-operative day (trials 1–3)		
			7	10	13
Mean preference ratio*	NAS	12	0.81 ± 0.06	0.90 ± 0.03	0.68 ± 0.04
	Control	12	0.63 ± 0.04	0.74 ± 0.05	0.75 ± 0.04
Drinking† (mean ml consumed per 15 min)	NAS	12	10.58 ± 0.52	12.04 ± 0.90	14.18 ± 1.04
	Control	12	9.13 ± 0.45	12.20 ± 0.63	12.61 ± 0.62
Locomotor activity‡ (quadrants crossed per 15 min)	NAS	12	41.2 ± 6.15	—	31.5 ± 5.33
	Control	12	71.6 ± 5.92	—	53.2 ± 2.71
Mean duration of a bout of drinking§ (s)	NAS	12	17.37 ± 2.29	—	21.79 ± 4.10
	Control	12	10.19 ± 1.39	—	14.47 ± 1.50

*The preference ratio was calculated for each rat by dividing volume consumed from the most preferred burette by the total volume consumed from all three burettes. Values thus range from 0.33 (no preference) to 1.00 (maximum preference for a single burette). The NAS group showed significant enhancement of preference on trials 1 and 2 ($F=8.19$, d.f.=1,22, $P<0.01$ and $F=7.30$, d.f.=1,22, $P<0.01$, respectively), following overall analysis of variance with repeated measures²⁴.

†There were no significant differences in water intake between the NAS and control groups $F<1.00$, $P>0.05$.

‡The NAS group was significantly less active than controls ($F=16.60$, d.f.=1,22, $P<0.01$).

§The NAS group exhibited significantly longer mean durations of a drinking bout ($F=4.95$, d.f.=1,22, $P<0.05$). Locomotor activity and duration of drinking were measured only on trials 1 and 3. The data from one rat were excluded from trial 3 because of a technical error.

in consummatory behaviour observed in rats with nigrostriatal DA damage¹⁶. Our rats with lesions to the region of the NAS, while showing no regulatory impairments, exhibit instead changes in nonspecific excitement engendered by a motivational state.

Second, only one other study has demonstrated by neural means a dissociation between 'displacement' and 'deficit' drinking²⁸. That experiment showed that hippocampal lesions, probably in conjunction with endocrine changes in the pituitary-adrenal axis, enhanced adjunctive drinking, while not significantly changing deprivation-induced drinking. Thus, these results support the contention that displacement behaviour may develop in part by effects of nonspecific motivational excitement, including stress.

Finally, the excessive and compulsive nature of displacement activities has not been ignored in the context of human mental disorder^{9,12,13,29,30}. The present experiments have linked the induction of one such displacement activity, in the rat, to a neuronal system already implicated in the behavioural effects of amphetamine²⁷, and of stress³¹ as well as in neurological³² and mental³³ dysfunction.

We acknowledge financial support from the UK Mental Health Trust Fund and USPHS grant AA03504 to Dr F. E. Bloom at Salk Institute. We thank Dr P. Emson and Ms J. Reed for performing the T-OH assays, Dr W. Shoemaker and Ms V. Sapin for measuring DA levels, R. E. Strecker for technical assistance, P. J. Fray and Dr B. J. Sahakian for critical advice and Professor O. L. Zangwill for research facilities.

Received 4 March; accepted 31 March 1980.

- McFarland, D. J. in *Advances in the Study of Animal Behaviour* Vol. 5 (eds Lehrman, D. S., Hinde, R. H. & Shaw, E.) 201–225 (Academic, London, 1974).
- Tinbergen, N. E. Q. *Rev. Biol.* **27**, 1–32 (1952).
- Hinde, R. A. *Animal Behaviour: a Synthesis of Ethology and Comparative Psychology* (McGraw-Hill, New York, 1966).
- Lindvall, O. & Bjorklund, A. in *Handbook of Psychopharmacology* Vol. 9 (eds Iversen, L. L., Iversen, S. D. & Snyder, S. H.) 139–231 (Plenum, New York, 1978).
- Fallon, J. H. & Moore, R. Y. *J. comp. Neurol.* **180**, 545–580 (1978).
- Simon, H., Le Moal, M., Gale, D. & Carado, B. *Brain Res.* **115**, 215–231 (1976).
- Falk, J. L. *Physiol. Behav.* **6**, 577–588 (1971).
- Staddon, J. E. R. & Simmelhag, V. L. *Psychol. Rev.* **78**, 3–43 (1971).
- Kachanoff, R., Leveille, R., McClelland, J. P. & Wayner, M. J. *Physiol. Behav.* **11**, 395–398 (1973).
- Killeen, P. R., Hanson, S. J. & Osbourne, S. R. *Psych. Rev.* **85**, 571–581 (1978).
- Wallace, M. & Singer, G. *Pharmac. Biochem. Behav.* **5**, 483–490 (1976).
- Wayner, M. J. *Physiol. Behav.* **5**, 1319–1325 (1970).
- Cantor, M. B. & Wilson, J. F. *Learn. Motiv.* **9**, 428–445 (1978).
- Falk, J. L. in *Thirst* (ed. Wayner, M. J.) 95–113 (Pergamon, New York, 1964).
- Teitelbaum, P. & Epstein, A. N. *Psych. Rev.* **69**, 74–90 (1962).
- Ungerstedt, U. *Acta physiol. scand. Suppl.* **367**, 95–122 (1971).

17. Koob, G. F., Riley, S. J., Smith, S. C. & Robbins, T. W. *J. comp. Physiol. Psychol.* **92**, 651–660 (1978).
18. Pellegrino, L. J. & Cushman, A. J. *A Stereotaxic Atlas of Rat Brain* (Appleton-Century-Crofts, New York, 1967).
19. Breese, G. R. & Traylor, T. D. *Br. J. Pharmac.* **42**, 88–89 (1972).
20. Coyle, J. T. & Henry, D. J. *Neurochem.* **21**, 61–67 (1973).
21. Saller, C. F. & Zigmond, M. J. *Life Sci.* **23**, 1117–1130 (1978).
22. Hendry, I. A. & Iversen, L. L. *Brain Res.* **29**, 159–162 (1971).
23. Sanger, D. J. & Blackman, D. E. in *Contemporary Research in Behavioural Pharmacology* (ed. Blackman, D. E. & Sanger, D. J.) 239–287 (Plenum, New York, 1978).
24. Winer, B. J. *Statistical Principles in Experimental Design* 2nd edn (McGraw-Hill, New York, 1971).
25. Jones, D. L. & Mogenson, G. J. *Eur. J. Pharmac.* **59**, 11–21 (1979).
26. Robbins, T. W. *Neurosci. Lett.* **S58**, Suppl. 1 (1978).
27. Kelly, P. H., Seviour, P. & Iversen, S. D. *Brain Res.* **94**, 507–522 (1975).
28. Devenport, L. D., *J. comp. physiol. Psychol.* **92**, 651–660 (1978).
29. Skinner, B. F. & Morse, W. H. *J. comp. physiol. Psychol.* **50**, 279–281 (1957).
30. Holland, H. C. in *Obsessional States* (ed. Beech, H. R.) 161–173 (Methuen, London, 1974).
31. Thierry, A. M., Tassin, J. P., Blanc, G. & Glowinski, J. *Nature* **263**, 242–244 (1976).
32. Price, K. A., Farley, I. J. & Hornykiewicz, O. in *Dopamine* (eds Rogers, P. J., Woodruff, G. N. & Iversen, L. L.) 293–300 (Raven, New York, 1978).
33. Stevens, J. R. *Archs gen Psychiat.* **29**, 177–189 (1973).

Genetic retrieval of viral genome sequences from herpes simplex virus transformed cells

M. Park, D. M. Lonsdale, M. C. Timbury,
J. H. Subak-Sharpe & J. C. M. Macnab

Institute of Virology, Church St, Glasgow G11 5JR, UK

Oncogenic transformation of cultured cells by inactivated herpes simplex virus (HSV) types 1 and 2 has been demonstrated^{1–12}. Expression of HSV information in these transformed cells has been shown by immunofluorescence studies^{2,6,8,13–15}, detection of HSV neutralizing antibody in sera from tumour-bearing animals^{1,4,7,11,12} and by hybridization of HSV-specific RNA^{16–18}. Molecular hybridization studies of DNA from HSV-2 transformed hamster cells have detected up to 40% of the HSV genome present in several copies^{16,19,20}. Complementation of three HSV-2 temperature-sensitive mutants when superinfecting the RE1 rat embryo cell line²¹ (transformed by the HSV-2 temperature-sensitive mutant *ts1*²²) suggests that resident viral genes can be expressed. Brown *et al.*²³ used a similar approach to detect HSV information latent in human ganglia. We report here retrieval of intertypic HSV recombinants from HSV transformed cells after superinfection with *ts* mutants of the alternative serotype of HSV. Restriction enzyme analysis which clearly differentiates between HSV-1 and HSV-2 DNA^{24,25} has demonstrated the isolation of recombinants spanning the genome and of virus indistinguishable from the original transforming virus.

Hooded Lister rat embryo cells transformed either by sheared DNA of HSV-1 strain α (RE α)⁷ or by an HSV-2 HG 52 mutant *ts1* (RE1) were used^{5,6}. Both transformed lines express HSV antigens²⁶ and HSV thymidine kinase activity²⁷.

Recombination and complementation were studied by comparing the virus yields from control (Hooded Lister rat embryo) and transformed (RE α , RE1) cells after superinfection

with a *ts* mutant with distinct genome characteristics. Control and transformed cells of equal density were superinfected with a *ts* mutant at a multiplicity of infection of 5 plaque forming units (PFU) per cell at both the permissive (31°C) and nonpermissive (38.5°C) temperatures. Twenty-four hours after infection virus produced by the control and the transformed cells was harvested. The yields were then titrated in BHK 21 cells at 38.5°C to identify putative *ts*⁺ recombinants and at 31°C to measure total yield; where the initial incubation was at 38.5°C this measures complementation.

ts⁺ virus (putative recombinants or revertants) was selected by picking separate plaques growing at 38.5°C from both control and transformed cell plates and taking each isolate through three successive plaque purification steps at 38.5°C (by picking well isolated plaques in each case). No virus picked from control cell plates survived this purification at 38.5°C. *ts*⁺ virus isolated was then labelled with ³²P-orthophosphate. After DNA extraction and restriction enzyme digestion according to Lonsdale²⁸, the fragment profiles were analysed by electrophoresis on agarose gels of appropriate concentration.

Analysis of the restriction enzyme profiles unambiguously identified a number of *ts*⁺ viruses as recombinants, some generated after superinfection of the RE1 cell line intertypically with HSV-1 17 *tsJ*, and some on superinfection of the RE α cell line intertypically with HSV-2 HG 52 *ts1* or intratypically with HSV-1, 17 *tsG* syn⁺.

Restriction enzyme analysis of the intertypic recombinants from RE1 cells $\times$ HSV-1 *tsJ* and from RE α cells $\times$ HSV-2 *ts1* allows detection and mapping of HSV-1 and HSV-2 sequences present. Figure 2 (right) gives the KpnI profile of 3 recombinants (8B, 6, 13) selected from 11 analysed, isolated after superinfection of RE α cells with HSV-2 *ts1*. Combined analysis of the *Bam*HI, *Kpn*I, *Hind*III and *Hpa*I restriction endonuclease profiles has yielded the genome structures shown in Fig. 3. Every recombinant clearly contains DNA sequences from the genome present in the transformed cells extending

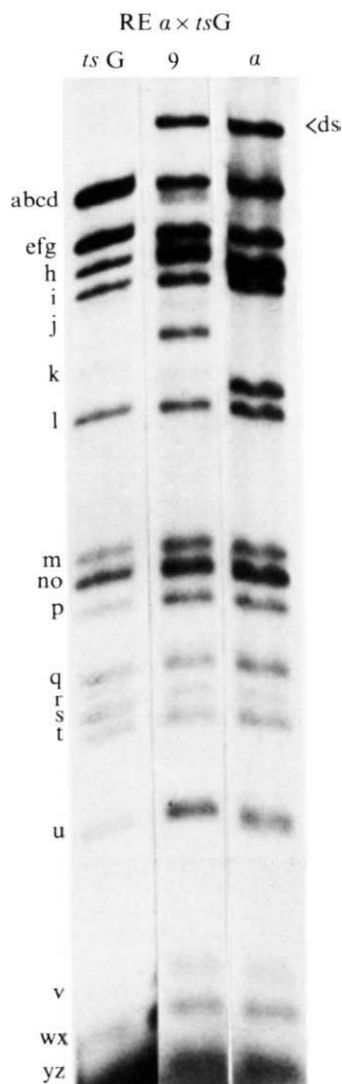


Fig. 1 Autoradiograph of ^{32}P -labelled DNA of HSV-1, HFEM α and one representative recombinant (no. 9) which were digested with restriction endonuclease *Kpn*I and subjected to electrophoresis on a 0.5% agarose gel. DNA fragments are lettered in accordance with the HSV-1 strain 17*Kpn*I restriction endonuclease map published previously³⁸. Those sequences characteristic of HSV-1 α at 0.54–0.65 map coordinates are detected by a *Kpn*I digest shown by the separate bands d and s of HSV-117 which are fused to give a new (d+s) larger molecular weight band characteristic of HSV-1 α . The d+s fusion of HSV-1 HFEM α is marked ds, the molar band present in the recombinant which corresponds to neither HSV-1 strain 17 or HSV-1 HFEM α represents an intermediate band resulting from the crossover event. The restriction enzyme profiles of the nine recombinants analysed resemble one another due to the similarities between the genome structures of HSV-1 HFEM α and HSV-117 analysis and definition of the crossover regions in these recombinants is not possible.

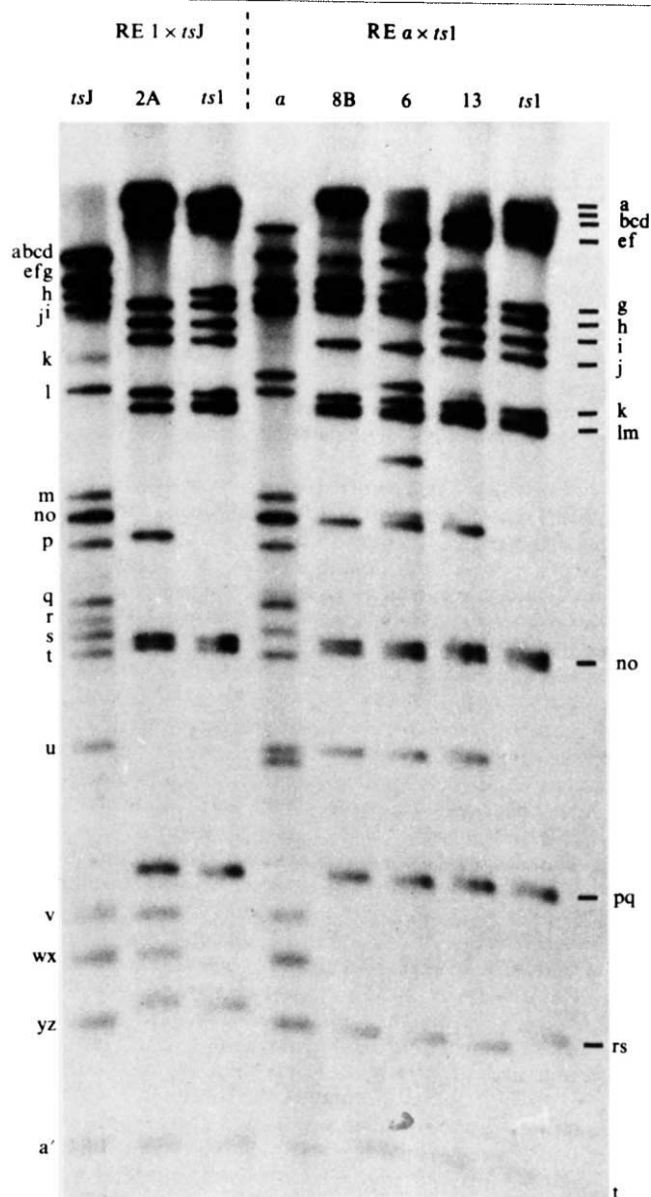


Fig. 2 Autoradiograph of ^{32}P -labelled DNAs of HSV-1 *tsJ*, HFEM α , HSV-2 *ts1* and recombinants 2A, 6, 8B, 13 which were digested with restriction endonuclease *Kpn*I and subjected to electrophoresis on an 0.8% agarose gel. DNA fragments are lettered in accordance with the HSV-117*Kpn*I restriction endonuclease map³⁸ and HSV-2 HG52 *Kpn*I restriction endonuclease map²⁴. Isolates 6, 8B, 13 are characteristic of recombinants analysed resemble one another, due to the HSV-2 *ts1*. The site of the *ts1* mutation is known to map at position 0.7 from marker rescue studies³⁹ and all recombinants produced after superinfection with *ts1* have a substitution of α DNA in this region, although each recombinant contains differing amounts of rescued information. Recombinant 2A isolated after superinfection of RE1 cell line with HSV-1 *tsJ* has a characteristic DNA profile of HSV-2 with an HSV-1 insertion between 0.38 and 0.50 map units. The restriction endonuclease profiles of these intertypic recombinants can clearly be related to bands originating from either the superinfecting virus or the original transforming virus. Bands which are not represented in either the superinfecting or the transforming virus are evidently intermediate bands formed as a result of the recombination event and could be identified by analysis of bands flanking the crossover region(s).

over a number of genes, some identified as coding for immediate early messages^{29–31}.

Recombinant 2A (Figs 2, 3) was isolated after superinfection of the RE1 cell line with HSV-1 *tsJ* and comprises ~90% HSV-2 sequences rescued from the RE1 cell line and only a small region (~10%) of DNA originating from the superinfecting virus.

Analysis of recombinants from the RE α cell line $\times$ HSV-2 *ts1* superinfection also produced one isolate with a restriction endonuclease profile indistinguishable from that of the original transforming virus HSV-1 α . This suggests that the sheared DNA of HSV-1 α used originally to produce the RE α

transformation may have contained some unshered molecules and that the entire HSV-1 α genome may be present in the RE α transformed cell line, but apparently not in the usual infectious state. Similarly, a putative recombinant from RE 1 cell line $\times$ HSV-1 *tsJ* superinfection was found to have a restriction endonuclease profile indistinguishable from HSV-2 *tsI*.

In the intratypic superinfection of the RE α cell line by *tsG* syn⁺, recombinants could be selected due to their altered plaque morphology. The syncytial plaque morphology marker of the transforming virus HSV-1 α and not that of the nonsyncytial superinfecting virus *tsG* syn⁺ was expressed by up to 25% of plaques grown at 38.5°C. Linkage of the *tsG* lesion to the plaque morphology locus has been demonstrated previously^{32,33}. Restriction enzyme analysis of nine separately isolated recombinants demonstrated retrieval of the HSV-1 α sequence between 0.54 and 0.65 map coordinates on the HSV-1 genome (Fig. 1). The similarities of the two HSV-1 genomes, however, make further characterization of the intratypic recombinants difficult; Fig. 3 (top) diagrammatically indicates the sequences arising unambiguously from HSV-1 α where recombinants have been isolated. Restriction enzyme analysis of HSV-1 strain α DNA has not shown any differences compared with HSV-1 strain HFEM from which it was derived (J. H. S.-S., unpublished). We shall, therefore, refer to HSV-1 α virus as HSV-1 HFEM α .

As expected from previous results²¹ several *ts* mutants are complemented by these transformed cell lines (Table 1). Intertypic and intratypic complementation is detected by elevated yields of *ts* superinfecting virus obtained at 38.5°C from the transformed cell line compared to the control cell line. The complementation is measured by titrating this yield at 31°C. All the *ts* mutants used are known to complement one another intratypically in standard genetic complementation tests³³.

Evidence from other laboratories suggests that up to 40% of the HSV genome is maintained in transformed hamster cells^{16,19,20}, although isolated restriction endonuclease fragments much smaller than this can initiate the morphological transformation of hamster cells (refs 34–36, and I. R. Cameron *et al.*, unpublished results). Heterogeneous populations of transformed cells, such as the uncloned RE 1 and RE α lines, can therefore contain the HSV genetic information required not only to initiate and/or to maintain the transformed state but also for many other viral functions.

The generation of recombinants (containing a large portion or apparently all the genome of the transforming virus) may have originated through successive recombination steps, either involving initial recombinants containing various regions of the HSV genome rescued from the transformed cells, or through recombinant virus again rescuing further information from the transformed cells. It is not yet known whether the genetic material of the transforming virus is present and

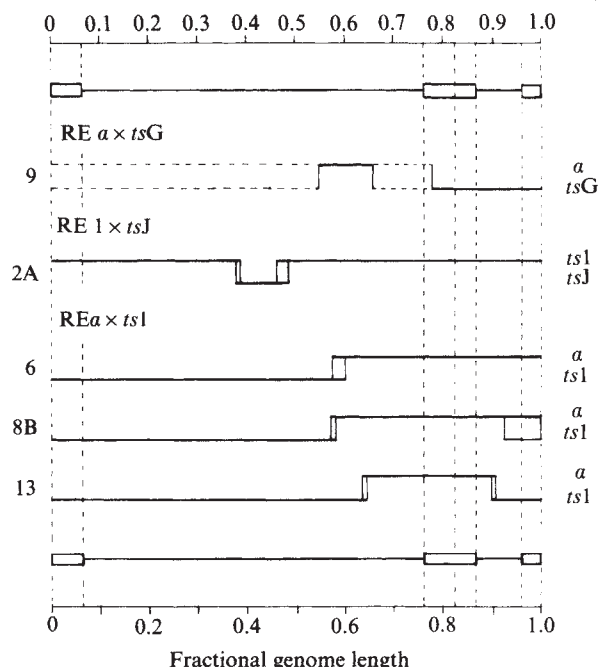


Fig. 3 Molecular model for the HSV genome⁴⁰ and analysis of crossover points in recombinants 9, 2A, 6, 8B, 13. The DNA sequences rescued from the transformed cell line form the uppermost line in each recombinant, whereas those present from the superinfecting virus compose the lower line of each structure. The box areas are those regions of uncertainty where a crossover event has occurred. The dotted lines in recombinant 9 indicate regions of uncertainty; due to similarities in restriction endonuclease profiles, HSV-1 *tsG* and HFEM α cannot be distinguished in these regions. The solid lines in this recombinant represent regions of the genome which can be identified as originating from either the superinfecting virus or the original transforming virus. Several restriction digests have revealed substantial differences between HFEM α and HSV-117 which show that the short unique region of these recombinants arises from the superinfecting virus HSV-1 *tsG*. Furthermore, HFEM α has a 2×10^6 dalton deletion in the internal long repeat compared to HSV-117: this is not represented in any of the recombinants allowing characterization of these sequences as arising from HSV-1 *tsG*.

maintained in the transformed cell in plasmid form or integrated into the chromosomal material of the cell; how many copies are present; or whether all viral sequences are present in equal amounts. These problems are being investigated.

If the HSV-DNA maintained in the transformed cell contains most of the HSV genome in a non-replicating form, infection by superinfecting virus may suffice to switch on the necessary pre-extant control systems³⁷, or provide a missing function which enables the resident genome to undergo normal replication. Once present in the replicating pool of virus genomes, recombination of the transforming virus DNA with the superinfecting *ts* mutant DNA would eventually produce *ts*⁺ recombinants.

This novel method of genetic information retrieval by superinfection with genetic probes demonstrates not only that recombinants spanning the genome may be isolated, but also that virus with the characteristic restriction enzyme pattern of the original transforming virus can be rescued after a large number of transformed cell generations.

We thank Dr I. Halliburton for unpublished maps of HSV-1 strain HFEM, Drs N. Frenkel, H. Locker and B. Roizman for communicating their unpublished maps, Dr S. M. Brown for mutants of HSV-1 strain 17 and Mr A. Orr for technical assistance.

Received 21 December 1979; accepted 2 April 1980.

Table 1 Complementation of *ts* mutants by transformed cell lines

Transformed cell line	Superinfecting <i>ts</i> mutant	Complementation index
RE α	HSV-1 <i>tsG</i>	39
	<i>tsI</i>	3.4
	<i>tsA</i>	13
	<i>tsD</i>	3.7
	HSV-2 <i>tsI</i>	7.7
RE 1	HSV-1 <i>tsJ</i>	31
	<i>tsI</i>	15
	<i>tsF</i>	6.5
	<i>tsA</i>	6

A complementation index ≥ 3 on titration of yields of *ts* superinfecting virus obtained at 38.5°C from the transformed cell line compared to the control cell line is regarded as evidence for complementation of the mutant virus by the transformed cell line.

1. Duff, R. & Rapp, F. J. *Virol.* **8**, 469-477 (1971).
2. Duff, R. & Rapp, F. J. *Virol.* **12**, 209-217 (1973).
3. Kutinova, L., Vonka, V. & Broucer, J. *J. natn. Cancer Inst.* **50**, 759-763 (1973).
4. Kimura, S., Flannery, V. L., Liez, B. & Schaffer, P. A. *Int. J. Cancer* **15**, 786-789 (1975).
5. Macnab, J. C. M. *J. gen. Virol.* **24**, 143-153 (1974).
6. Macnab, J. C. M. in *2nd Int. Symp. Oncogenesis and Herpesviruses* Vol. 1, 227-235 (International Agency for Research on Cancer, Lyon, 1975).
7. Wilkie, N. M., Clements, J. B., Macnab, J. C. M. & Subak-Sharpe, J. H. *Cold Spring Harb. Symp. quant. Biol.* **39**, 657-666 (1974).
8. Darai, G. & Munk, K. *Int. J. Cancer* **18**, 469-481 (1976).
9. Darai, G., Braun, R., Flugel, R. M. & Munk, K. *Nature* **265**, 744-746 (1977).
10. Kucera, L. S., Gudson, J. P., Edwards, I. & Herbst, G. J. *gen. Virol.* **35**, 473-485 (1977).
11. Boyd, A., Orme, T. & Boone, C. *2nd Int. Symp. Oncogenesis and Herpesviruses* Vol. 1, 429-439 (International Agency for Research on Cancer, Lyon, 1975).
12. Boyd, A. & Orme, T. W. *Int. J. Cancer* **16**, 526-538 (1975).
13. Flannery, V. L., Courtney, R. J. & Schaffer, P. A. *J. Virol.* **21**, 284-291 (1977).
14. Gupta, P. & Rapp, F. *Proc. natn. Acad. Sci. U.S.A.* **74**, 372-374 (1977).
15. Reed, C. L., Cohen, G. H. & Rapp, F. J. *Virol.* **15**, 668-670 (1975).
16. Kessous, A., Bibor-Hardy, V., Sult, M. & Simard, R. *Cancer Res.* **39**, 3225-3234 (1979).
17. Collard, W., Thornton, H. & Green, M. *Nature new biol.* **243**, 264-265 (1973).
18. Copple, C. D. & McDougall, J. K. *Int. J. Cancer* **17**, 501-513 (1976).
19. Minson, A. C., Thoulous, M. E., Eglin, R. O. & Darby, G. *Int. J. Cancer* **17**, 493-500 (1976).
20. Frenkel, N., Locker, H., Cox, B., Roizman, B. & Rapp, F. J. *Virol.* **18**, 885-893 (1976).
21. Macnab, J. C. M. & Timbury, M. C. *Nature* **261**, 233-235 (1976).
22. Timbury, M. C. *J. gen. Virol.* **13**, 373-376 (1971).
23. Brown, S. M., Subak-Sharpe, J. H., Warren, K. G., Wroblewska, Z. & Koprowski, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2364-2368 (1979).
24. Morse, L. S., Buchan, G., Roizman, B. & Schaffer, P. A. *J. Virol.* **24**, 231-248 (1977).
25. Wilkie, N. M. et al. *3rd Int. Symp. Oncogenesis and Herpesviruses* Vol. 1, 11-33 (International Agency for Research on Cancer, Lyon, 1978).
26. Macnab, J. C. M. *J. gen. Virol.* **43**, 39-56 (1979).
27. Macnab, J. C. M., Visser, L., Jamieson, A. T. & Hay, J. *Cancer Res.* (in the press).
28. Lonsdale, D. M. *Lancet* **i**, 849-851 (1979).
29. Clements, J. B., Watson, R. J. & Wilkie, N. M. *Cell* **12**, 275-285 (1977).
30. Marsden, H. S., Stow, N. D., Preston, V. G., Timbury, M. C. & Wilkie, N. M. *J. Virol.* **28**, 624-642 (1978).
31. Clements, J. B., McLauchlan, J. & McGeoch, D. J. *Nucleic Acids Res.* **7**, 77-91 (1979).
32. Ritchie, D. A., Brown, S. M., Subak-Sharpe, J. H. & Jamieson, A. J. *Virology* **82**, 323-333 (1977).
33. Brown, S. M., Ritchie, D. A. & Subak-Sharpe, J. H. *J. gen. Virol.* **18**, 329-346 (1973).
34. Camacho, A. & Spear, P. G. *Cell* **15**, 993-1002 (1978).
35. Reyes, G. R., La Femina, R., Hayward, S. D. & Hayward, G. S. *Cold Spring Harb. Symp. quant. Biol.* **44** (1979).
36. Jariwalla, R. J. & Aurelian, L. *Proc. Natn. Acad. Sci. U.S.A.* (in the press).
37. Leiden, J. M., Buttyan, R. & Spear, P. C. *J. Virol.* **20**, 413-424 (1976).
38. Preston, V. G. et al. *J. Virol.* **28**, 499-517 (1978).
39. Wilkie, N. M. et al. *Cold Spring Harb. Symp. quant. Biol.* **43**, 827-840 (1978).
40. Sheldrick, P. & Berthelot, N. *Cold Spring Harb. Symp. quant. Biol.* **39**, 667-678 (1974).

A novel human pituitary peptide containing the γ -MSH sequence

S. Benjannet, N. G. Seidah, R. Routhier & M. Chrétien

Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal H2W 1R7, Canada

It is well established that ACTH and β -lipotropin (LPH) originate from a common precursor molecule¹⁻³. Recently the complete complementary DNA sequence of the bovine precursor was reported⁴, and within the cryptic sequence of this molecule is a third melanocyte stimulating hormone (MSH) region tentatively named γ -MSH⁴. The signal peptide of this molecule consists of 26 amino acids in both the rat⁵ and mouse⁶. Pulse-chase experiments using both rat and mouse pituitary cells, showed the gradual maturation of this common precursor to proceed via the initial cleavage of the carboxy terminal β -LPH, followed by release of ACTH, leaving an NH₂-terminal extension of about 105 amino acids, which does not seem to undergo appreciably further maturation^{1-3,7}. It is within the sequence of this NH₂-terminal extension that γ -MSH is located⁴. It is not yet clear what the biological role of this molecule is and whether γ -MSH itself is released. Recently, it was shown that a synthetic 12 amino acid bovine γ -MSH fragment possessed very little melanophore-stimulating activity as compared to α -MSH⁸. We report here the successful purification of the human NH₂ terminal cryptic peptide, its amino acid composition and present some of its tryptic fragments. The data show that human and bovine γ -MSH have identical amino acid composition.

Starting from an HCl/acetone extract of frozen human pituitaries, an initial ion-exchange fractionation on CM-cellulose was performed as previously described for the purification of human β -LPH and β -endorphin⁹. The unretained peak was further fractionated by gel chromatography on Sephadex G-75. Labelling the peptides obtained from the various fractions with ¹⁴C-iodoacetamide and microsequencing allowed the identification of a fraction enriched with a peptide showing the sequence Cys-2, 8, 20 and 24. There are exactly the cysteine positions that would be expected for the bovine ACTH/LPH

Fig. 1 Comparison of the reported bovine sequence of γ -MSH⁴ within the NH₂-terminal ACTH/LPH segment to that deduced from the amino acid composition of homologous human tryptic fragments. T5 is a minor chymotryptic fragment isolated.

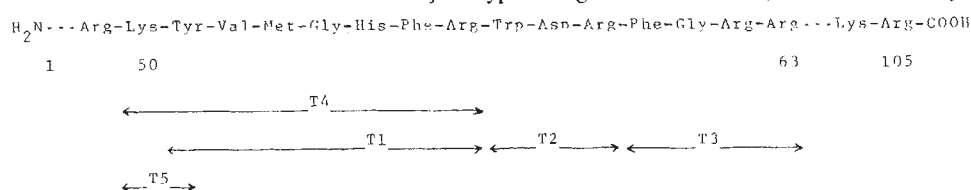


Table 1 Amino acid composition of human tryptic peptides corresponding to γ -MSH

Amino acid	T1	T2	T3	T4	T5
Trp	—	0.54 (1)	—	—	—
Lys	—	—	—	0.79 (1)	1.10 (1)
His	1.02 (1)	—	—	0.97 (1)	—
Arg	1.11 (1)	1.06 (1)	1.96 (2)	1.09 (1)	—
SCM-Cys	—	—	—	—	—
Asp	—	0.98 (1)	—	—	—
Thr	—	—	—	—	—
Ser	—	—	—	—	—
Glu	—	—	—	—	—
Pro	—	—	—	—	—
Gly	1.32 (1)	—	0.84 (1)	1.39 (1)	—
Ala	—	—	—	—	—
Val	0.90 (1)	—	—	1.07 (1)	—
Met	0.78 (1)	—	—	0.80 (1)	—
Ile	—	—	—	—	—
Leu	—	—	—	—	—
Tyr	1.04 (1)	—	—	0.93 (1)	0.92 (1)
Phe	0.93 (1)	—	1.20 (1)	1.01 (1)	—

The human pituitary peptide (1 mg) was subjected to tryptic digestion. Peptides were separated in a μ -bondapak C₁₈ HPLC column and purified for amino acid analysis. The data represent 24-h hydrolysates analysed on a Beckman 121M amino acid analyser. The values in parentheses represent the values expected from the bovine γ -MSH sequence reported⁴.

precursor had the 26 residues signal peptide been cleaved off^{5,6}. Further purification by HPLC on a Waters μ -bondapak C₁₈ column¹⁰ allowed the isolation of 5 mg of a pure polypeptide bearing identical cysteine residues sequence positions as the bovine homologue. On SDS-polyacrylamide gel electrophoresis it was homogeneous and migrated with an apparent molecular weight of 18,000. This peptide is not recognized by an ACTH₍₁₋₂₄₎ directed antibody⁵ and hence lacks the ACTH region of the ACTH/LPH precursor.

The total amino acid composition of the purified human peptide was: Trp 2, Lys 3, His 2, Arg 8, Cys 4, Asp 11, Thr 5, Ser 12, Glu 13, Pro 8, Gly 11, Ala 6, Val 2, Met 2, Ile 1, Leu 8, Tyr 1 and Phe 4. This gives a total of 103 amino acids compared to a maximum of 105 for the bovine homologue⁴. The compositions of four major tryptic fragments (T1 to T4) and a minor chy-

motryptic one (T5) are shown in Table 1. In Fig. 1, the alignment of these human peptides with the reported bovine γ -MSH sequence is shown. The reported bovine γ -MSH sequence, Lys-Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-Arg-Arg fits exactly the amino acid compositions obtained from the human tryptic fragments.

An additional indication that this region of the molecule is identical in human and cattle is that peptides T1, T2 and T3 co-eluted on the HPLC chromatogram with their corresponding synthetic bovine homologues. Furthermore, an antibody raised against the bovine γ -MSH sequence, which does not recognize β -LPH, ACTH, α -MSH or β -MSH¹¹, showed complete cross reactivity on a molar basis for the human NH₂-terminal peptide purified as compared to the synthetic bovine γ -MSH peptide.

The finding of apparently identical structures of human and bovine γ -MSH regions emphasizes the conservation of the structure of this segment of the ACTH/LPH precursor between these species. Similar observations have been made for the species sequence homologies of the α -MSH, β -MSH and β -endorphin regions of this precursor. Although no distinct biological activity of the NH₂-terminal segment of the ACTH/LPH precursor has been found, the structural homologies suggest that this segment may have an as yet undiscovered function. The purification of a human peptide of MW 18,000 related to the NH₂-terminal segment of the ACTH/LPH precursor, in which the ACTH molecule is absent, indicates that the mode of maturation of the ACTH/LPH precursor in the human pituitary could be similar to that observed in other species such as the rat and mouse¹⁻³.

Since submission of this paper, Hakanson *et al.* have described the isolation of a similar peptide from pig pituitary¹².

This work was supported by grant PG-2 from the MRC of Canada.

Received 4 February; accepted 17 March 1980.

1. Roberts, J. L. & Herbert, E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5300-5304 (1977).
2. Mains, R. E., Eipper, B. A. & Ling, N. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3014-3018 (1977).
3. Crine, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4719-4723 (1978).
4. Nakanishi, S. *et al. Nature* **278**, 423-427 (1979).
5. Gossard, F., Seidah, N. G., Crine, P., Routhier, R. & Chrétien, M. *Biochem. biophys. Res. Commun.* **92**, 1042-1051 (1980).
6. Keutmann, H. T., Eipper, B. A. & Mains, R. E. *J. biol. Chem.* **254**, 9204-9208 (1979).
7. Gianoulakis, C., Seidah, N. G., Routhier, R. & Chrétien, M. *J. biol. Chem.* **254**, 11903-11906 (1979).
8. Ling, N., Ying, S., Minick, S. & Guillemin, R. *Life Sci.* **25**, 1773-1780 (1979).
9. Chrétien, M., Benjannet, S., Dragon, N., Seidah, N. G. & Lis, M. *Biochem. biophys. Res. Commun.* **72**, 472-478 (1976).
10. Seidah, N. G. *et al. J. Chromatogr.* (in the press).
11. Seidah, N. G., Benjannet, S., Lis, M. & Chrétien, M. (in preparation).
12. Hakanson, R., Eckman, R., Sundler, F. & Nilsson, R. *Nature* **283**, 789-792 (1979).

Pituitary immunoreactive γ -melanotropins are glycosylated oligopeptides

Tamotsu Shibasaki, Nicholas Ling & Roger Guillemin

Laboratories for Neuroendocrinology, The Salk Institute for Biological Studies, La Jolla, California 92037

Nakanishi *et al.*¹ have recently characterised the complete sequence of the mRNA isolated from the intermediate lobe of bovine pituitary which codes for the 31,000 molecular weight (31 K) precursor protein of corticotropin/ β -lipotropin (ACTH/ β -LPH). The corresponding amino acid sequence translated from this mRNA revealed in the cryptic region of the precursor protein a fragment sharing a common amino acid sequence with the α - β -melanotropins (α -MSH, β -MSH) and thus named γ -MSH¹. To study whether this γ -MSH fragment is also processed and released as a biologically active substance and to ascertain its location in the pituitary and possibly in the brain, we have raised antibodies to the synthetic replicate of γ_3 -MSH (ref. 2). We report here the detection of at least two γ -MSH-like peptides in the pituitary using these antibodies in a radioimmunoassay (RIA) and, furthermore, evidence that these two peptides are glycosylated.

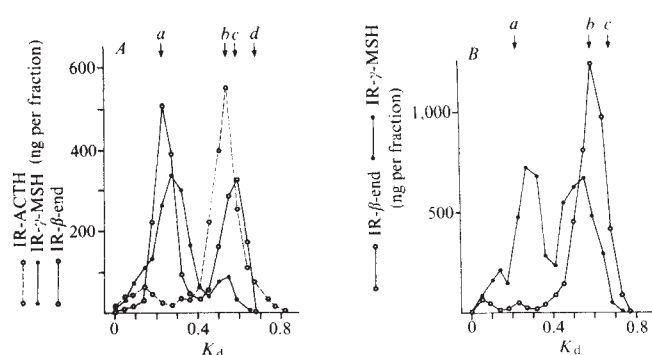


Fig. 1 Biogel P-100 gel permeation chromatography of the anterior (A) and intermediate (B) lobe extracts of bovine pituitary. For experimental conditions see text. Markers: a = ¹²⁵I-labelled β -LPH; b = ACTH; c = ¹²⁵I-labelled β -endorphin; d = ¹²⁵I-labelled γ_3 -MSH.

The antiserum raised in rabbits was obtained by injecting the synthetic replicate of γ_3 -MSH coupled to bovine serum albumin through bis-diazotised benzidine¹². The amino acid sequence of γ_3 -MSH as derived from Nakanishi *et al.*¹ is Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-Arg-Arg-Asn-Gly-Ser-Ser-Ser-Ser-Gly-Val-Gly-Gly-Ala-Ala-Gln-OH. The peptide was prepared by solid phase synthesis and obtained in high purity using methods already described². The antiserum raised against this peptide reads the portion between His 5 and Arg 14 without significant cross-reaction with α - and β -MSH, β -endorphin, β -LPH, corticotropin-like intermediate lobe peptide and ACTH.

Bovine pituitaries were obtained at a local abattoir, immediately frozen on dry ice, and kept in a freezer at -70 °C until use. After defrosting, a pituitary was carefully dissected into its three lobes. After cutting into small pieces, a 978-mg fragment of the anterior lobe and a 157-mg fragment of the intermediate lobe were extracted by Polytren in 20 ml and 8 ml, respectively, of cold 1 M acetic acid containing 20 mM HCl, 0.01% phenylmethylsulphonyl fluoride and 130 kIU ml⁻¹ Trasylol. The homogenates were centrifuged at 2,500g at 4 °C for 30 min and the supernatants lyophilized. The lyophilized materials from the anterior and intermediate lobes were reconstituted in 1.9 ml and 0.5 ml, respectively, of 4 M guanidine-HCl for gel permeation chromatography. A 0.2-ml aliquot of that solution was applied to a 0.7 × 48 cm Biogel P-100 column equilibrated in 4 M guanidine-HCl and eluted with the same solvent at 1.3 ml h⁻¹ at room temperature. The column was calibrated using dextran blue, ¹²⁵I-labelled ovine β -LPH, ovine ACTH, ¹²⁵I-porcine β -endorphin, ¹²⁵I-labelled γ_3 -MSH and phenol red. Fractions eluted from the column were diluted with the buffer for RIA of γ -MSH, ACTH and β -endorphin³. Final guanidine-HCl concentration in the RIA was 7.4 mM. An equivalent amount of guanidine-HCl was added to the tubes for preparing the RIA standard curves.

Figures 1A and B show the gel permeation chromatography of the anterior and intermediate lobe extracts as above. Two peaks with γ -MSH-like immunoreactivity in the anterior lobe extract, and two main peaks and one smaller peak with γ -MSH-like immunoreactivity in the intermediate lobe extract were detected with the γ -MSH RIA. The elution volume of the two peaks from the anterior lobe extract matches that of the latter two peaks from the intermediate lobe extract. The peak at K_d = 0.27 is much larger than that at K_d = 0.55 in the anterior lobe extract, whereas these two peaks show almost the same distribution in the intermediate lobe extract. These results suggest a difference in the post-translational processing of γ -MSH-like peptides in the anterior and intermediate lobe as is also the case in the processing of β -LPH, β -endorphin and ACTH: in the anterior lobe ACTH and β -LPH are the major products and in the intermediate lobe β -endorphin is predominant, with hardly any β -LPH or ACTH⁴.

The elution profile also shows that the molecular size of the native γ -MSH-like peptides is larger than that of the iodinated synthetic γ_3 -MSH with apparent molecular weights (MWs) of 13,000, 8,800 and 4,500 for the peaks at $K_d=0.14$, 0.27 and 0.55, respectively. A possible explanation for the larger MW may be that native γ -MSH-like peptides contain more than the 27 amino acids of γ_3 -MSH as derived from Nakanishi *et al.*¹. Another explanation is that native γ -MSH-like peptides may contain carbohydrate; indeed, it has been reported that in mouse ACTH-secreting pituitary tumours, the 16 K fragment of the 31 K ACTH/ β -LPH precursor protein, which should contain the γ -MSH region, is glycosylated². Both possibilities may account for the higher molecular form of native γ -MSHs.

The fractions corresponding to the two immunoreactive γ -MSH peaks in the anterior lobe extract and the three peaks in the intermediate lobe extract of bovine pituitary were examined with affinity chromatography on concanavalin A (Con A) coupled to Sepharose 4-B. As shown in Fig. 2, all the immunoreactive (IR-) γ -MSHs from both the anterior and intermediate pituitary are glycopeptides; all were retarded on the Con A column and could only be displaced with α -methyl-D-mannopyranoside (α -MM).

In the post-translational processing of the murine ACTH/ β -LPH precursor³, glycosylation occurs in the ACTH fragment and the resulting glycopeptide is called 13 K ACTH; glycosylation also occurs in the non-ACTH/ β -LPH portion of the precursor to give the 16 K fragment. In general, the asparagine residue within the amino acid sequence Asn-X-Thr (or Ser) is the site to which a carbohydrate chain is linked

through an *N*-acetylglucosamine⁷. However, it was proposed⁶ that the aspartic acid residue at the 29th amino acid position of ACTH₁₋₃₉ is the most likely site for attachment of a carbohydrate chain in the murine 13 K ACTH fragment. In the case of the γ -MSH-like peptides, the amino acid sequence Asn-X-Ser corresponds to residues 15-17 of γ_3 -MSH, and could be a likely site for glycosylation. However, native γ -MSHs may contain more than one carbohydrate chain as the Asn 15-X-Ser 17 sequence is followed by Ser 18-Ser-Ser in γ_3 -MSH. This series of serine residues could also be candidates to which oligosaccharides are linked through an *O*-glycosidic linkage⁸.

Post-translational glycosylation of the ACTH/ β -LPH precursor may protect it from intracellular degradation by providing it with specific conformational stability⁹. Glycosylation of native γ -MSHs may similarly have a role in extending their biological half life. Further studies are in progress to determine the amino acid sequence of the γ -MSHs and to characterise their carbohydrate moieties.

After submission of this report, Hakanson *et al.*¹⁰ described the isolation from extracts of porcine pituitaries of a large glycopeptide (103 amino acids), a partial sequence of which showed it to be the whole *N*-terminal region of the 31 K precursor molecule down to the starting point of ACTH. The present report shows that this large fragment is further processed to the smaller γ_3 -MSH. Furthermore, we have recent evidence that an even smaller fragment corresponding to γ_1 -MSH is also present in pituitary extracts and released with other fragments of the 31 K precursor.

We thank Dr S. Lavielle for helpful discussion and Mrs B. Gayer for secretarial help. The research was supported by NIH grants AM-18811-05 and HD-09690-05 and the William Randolph Heart Foundation. T.S. is a Postdoctoral fellow from the Department of Internal Medicine, Tokyo Women's Medical College, Tokyo, Japan.

Received 20 January; accepted 2 April 1980.

1. Nakanishi, S. *et al.* *Nature* **278**, 423-427 (1979).
2. Ling, N., Ying, S., Minick, S. & Guillemin, R. *Life Sci.* **25**, 1773-1780 (1979).
3. Shibasaki, T., Deftos, L. & Guillemin, R. *Biochem. biophys. Res. Commun.* **90**, 1266-1273 (1979).
4. Scott, A. P. *et al.* in *Peptide Hormones* (ed. Parsons, J. A.) 247-271 (University Park Press, Baltimore, 1976).
5. Eipper, B. A. & Mains, R. E. *J. biol. Chem.* **253**, 5732-5744 (1978).
6. Eipper, B. A. & Mains, R. E. *J. biol. Chem.* **252**, 8821-8832 (1977).
7. Sox, H. C. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **66**, 975-982 (1970).
8. Bhavanandan, V. P., Buddecke, E., Carabell, R. & Gottschalk, A. *Biochem. biophys. Res. Commun.* **16**, 353-357 (1964).
9. Loh, Y. P. & Gainer, H. *Endocrinology* **105**, 474-487 (1979).
10. Hakanson, R., Ekman, R., Sundler, F. & Nilsson, R. *Nature* **283**, 789-792 (1980).
11. Eipper, B. A., Mains, R. E. & Guenzi, D. J. *J. biol. Chem.* **251**, 4121-4126 (1976).
12. Shibasaki, T., Ling, N. & Guillemin, R. *Life Sci.* **26**, 1781-1785 (1980).

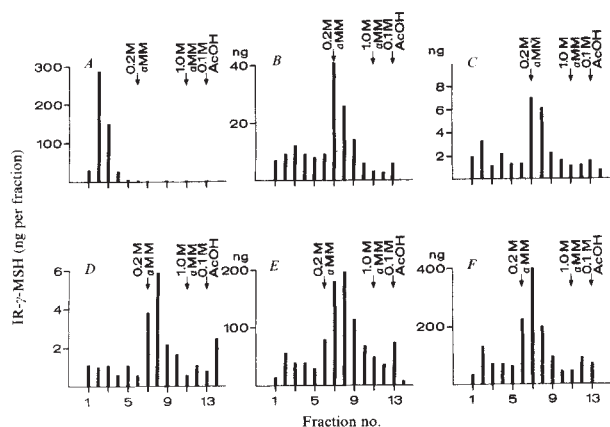


Fig. 2 Affinity chromatography of γ -MSH-like peptides on Con A-coupled Sepharose 4-B. A 1-ml siliconised glass syringe was packed with 0.4 ml Con A coupled to Sepharose 4-B. After being washed with 3 ml 0.1 M acetic acid, the column was equilibrated with Con A buffer composed of 0.01 M Tris-HCl, 0.7 mM $MgCl_2$, 0.1% bovine serum albumin, 1.0 M NaCl and 0.1% Triton X-100 (pH 7.4)¹¹. γ -MSH-like peptides for affinity chromatography were obtained by applying the anterior and intermediate lobe extracts of bovine pituitary, respectively, to a Sephadex G-75 column in 1.0 M acetic acid. The elution profiles of the two extracts were almost the same as that in the Biogel P-100 column chromatography in 4 M guanidine-HCl. The peak tubes corresponding to the elution volume at $K_d=0.27$ and 0.55 in the anterior lobe extract and at $K_d=0.14$, 0.27 and 0.55 in the intermediate lobe extract were lyophilised. The residue reconstituted in 0.5 ml of Con A buffer was applied to the column. After washing the column with 4.5 ml of the same buffer, 5 ml of 0.2 M α -methyl-D-mannopyranoside (α -MM) in Con A buffer was used to elute the retarded material, followed by 1.0 M α -MM and 0.1 M AcOH. Fractions (1 ml) were collected, lyophilised and the lyophilised fractions were reconstituted in buffer for RIA of γ -MSH. Recovery following elution with 0.2 M α -MM was 51-65% of applied IR- γ -MSH. A, Synthetic γ_3 -MSH; B, C, native γ -MSHs corresponding to $K_d=0.27$ and 0.55, respectively, of the anterior lobe extract; D, E, F, native γ -MSHs corresponding to $K_d=0.14$, 0.27 and 0.55, respectively, of the intermediate lobe extract.

Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides

Kazuhiko Tatemoto & Viktor Mutt

Department of Biochemistry II, Karolinska Institute, S-104 01 Stockholm 60, Sweden

Naturally occurring peptides with biological actions have in most cases been detected by observing their biological activities in crude extracts and their isolation has been followed using bioassays. As a complement to the classical biological detection systems, we have proposed a chemical detection system based on fragmentation of peptides in tissue extracts followed by identification of certain of these peptide fragments having distinct chemical features^{1,2}. One such chemical feature is the C-terminal amide structure which is characteristic of many

biologically active peptides^{3,4}. We have devised a chemical assay method for peptides having such a structure and have found several previously unknown peptide amides in porcine upper small intestinal tissues¹. We report here the isolation and characterization of two of them, designated PHI and PYY. PHI is related to secretin, vasoactive intestinal polypeptide (VIP), glucagon and gastric inhibitory polypeptide (GIP); PYY is related to the pancreatic polypeptide and to neurotensin. Both peptides exhibit biological activities and appear to be present not only in the intestine but also in brain.

The presence of peptides with the amidated C-terminal structure can be detected in crude extracts using a chemical assay in which the amidated C-terminal portion of the peptide chain is cleaved off enzymatically, converted into the fluorescent dansyl derivative and then selectively isolated and identified by TLC¹. In the present work, the levels of PHI or PYY in the tissue samples were estimated by measuring the concentration of isoleucine amide or tyrosine amide which was cleaved off from PHI or PYY, respectively, by degradation of the samples with thermolysin.

The starting material for the isolation work was a side fraction obtained during the purification of porcine secretin⁵. Boiled pig intestines (the uppermost 1 m of the small intestine) were extracted with 0.5 M acetic acid and the extracted peptides were adsorbed to alginic acid. They were then eluted from the alginic acid with 0.2 M HCl and precipitated with NaCl at saturation. The peptide concentrates were further purified by extraction with 66% ethanol, gel filtration (Sephadex G-25) and extraction with methanol⁵. The methanol-soluble fraction (after removal of the methanol) was subjected to ion-exchange chromatography on CM-cellulose (CMC) and PHI and PYY were found in a fraction eluting before secretin. This fraction was rechromatographed on CMC to separate PHI from PYY. The fractions containing PHI were further purified by gel filtration (Sephadex G-25), chromatography on DEAE-cellulose, isoelectric precipitation at pH 7.0 and finally by HPLC on the reversed phase, Bondapak C-18. The fractions containing PYY were further purified by gel filtration (Sephadex G-25), chromatography on CMC and finally by HPLC on Bondapak C-18. The final products were found to be homogenous by analysis with TLC, gel electrophoresis, HPLC, and by amino acid determinations.

The N-terminal analysis revealed the presence of histidine for PHI and tyrosine for PYY. Since the biological functions of these newly-isolated peptides are not yet established, we decided to give chemical names to them based on their N- and C-terminal amino acids (one-letter system). PHI thus designates 'the peptide (P) having N-terminal histidine (H) and C-terminal isoleucine (I) amide' and PYY designates 'the peptide (P) having N-terminal tyrosine (Y) and C-terminal tyrosine (Y) amide'.

PHI consists of 27 amino acids: 2 Ala, 1 Arg, 2 Asx, 2 Glx, 2 Gly, 1 His, 1 Ile, 5 Leu, 2 Lys, 2 Phe, 4 Ser, 1 Thr, 1 Tyr, 1 Val. A preliminary sequence analysis revealed the N-terminal sequence of His-Ala-Asp-Gly-Val-Phe-Thr-. The C-terminal sequence, -Leu-Ile-NH₂, was determined by the amide

```

PYYb      Y P A K P E A P G ----- R Ya
BPPc      A P L E P Q Y P G D D A T P E Q M A Q Y A A E L R R Y I N M L T R P R Ya
APPd      G P S Q P T Y P G D A P V E D L I R F Y D N I Q Q Y L N V V T R H R Ya
NEUROTENSINe Z L Y E N K P R R P Y I L

```

Fig. 2 The partial sequence of PYY and the comparison with the pancreatic polypeptide and neurotensin. a, The carboxyl end is amidated. b, The peptide (P) with N-terminal tyrosine (Y) and C-terminal tyrosine (Y). c, The porcine pancreatic polypeptide. d, The avian pancreatic polypeptide. e, The N-terminal amino acid is pyroglutamic acid.

identification method¹. The terminal sequences were compared with those of the secretin-glucagon family (Fig. 1) and remarkable sequence similarities were seen between PHI and secretin, VIP, glucagon and GIP, indicating that PHI probably belongs to this peptide group. PHI activated adenylate cyclase in various rat membrane systems and inhibited VIP binding to its receptors⁶. It was also found that PHI induced insulin release from an isolated rat pancreas at basal and elevated glucose levels, while it enhanced glucagon release in the presence of arginine⁷.

PYY consists of 36 amino acids: 4 Ala, 4 Arg, 2 Asx, 5 Glx, 1 Gly, 1 His, 4 Leu, 1 Lys, 4 Pro, 3 Ser, 1 Thr, 5 Tyr, 1 Val. The N-terminal sequence was found to be Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly. Treatment of PYY with trypsin yielded tyrosine amide. Since the only lysine residue in the molecule is found near the N-terminus, the C-terminal sequence should be -Arg-Tyr-NH₂, the same as that of the pancreatic polypeptide^{8,9}. The partial sequence of PYY was therefore compared with the sequence of the porcine and avian pancreatic polypeptides (Fig. 2). In addition to the distinct sequence homology to the pancreatic polypeptide, PYY possesses sequence similarities to neurotensin¹⁰ (Fig. 2). PYY was found to inhibit the action of secretin on the pancreas of the anaesthetized cat, the bicarbonate secretion induced by secretin in a single intravenous dose (0.2 µg per kg) being reduced to about 30% in the presence of PYY (5 µg per kg) (data not shown). In a dose range of 0.5–5 µg per kg, PYY contracted the gallbladder of the anaesthetized guinea pig in a dose-responsive fashion. Further studies on the biological properties of PYY are under way.

Preliminary studies indicate that there are unknown peptide amides in brain as well as in intestine. Judging from the chromatographic patterns, solubility properties, and the C-terminal amide determinations, it seems highly probable that both PHI and PYY are present and that PYY may constitute one of the major peptide amides of the brain.

This work was supported by the Swedish Medical Research Council through grant 13X-01010.

Received 26 November 1979; accepted 1 April 1980.

```

PHIb      H A D G V F T ----- La
SECRETIN  H S D G I F T S E L S R I R D S A R L Q R L L Q G L Ya
VIPc      H S D A V I T D N Y I R L R K Q N A V K K Y I N S I L Na
GLUCAGON  H S Q G I F T S D Y S K Y L D S R R A Q D F V Q W I M N T
GIPd      Y A E G T I S D Y S J A M D K I R Q Q D F V H W L L A Q Q K G K K S D W K H N I T O

```

Fig. 1 The partial sequence of PHI and the comparison with peptides of the secretin-glucagon family. a, The carboxyl end is amidated. b, The peptide (P) with N-terminal histidine (H) and C-terminal isoleucine (I). c, The vasoactive intestinal peptide (porcine). d, The gastric inhibitory peptide (porcine).

1. Tatemoto, K. & Mutt, V. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4115–4119 (1978).
2. Tatemoto, K. & Mutt, V. *Scand. J. Gastroenterol.* **13**, Suppl. 49, 181 (1978).
3. Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5 (ed. Dayhoff, M. O.) D173-D227 (National Biochemical Research Foundation, Silver Spring, Maryland, 1972).
4. Hunt, L. T. & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. II (ed. Dayhoff, M. O.) 113–145 (National Biochemical Research Foundation, Silver Spring, Maryland, 1976).
5. Mutt, V. in *Gut Hormones* (ed. Bloom, S. R.) 21–27 (Churchill-Livingstone, Edinburgh, 1978).
6. Bataille, D. et al. *Scand. J. Gastroenterol.* **13**, Suppl. 49, 13 (1978).
7. Szczcowska, J., Tatemoto, K., Mutt, V. & Efendić, S. *Life Sci.* **26**, 435–438 (1980).
8. Li, T.-M. & Chance, R. E. *Gastroenterology* **67**, 737–738 (1974).
9. Chance, R. E., Johnson, M. G., Hoffman, J. A. & Lin, T.-M. in *Proinsulin, Insulin, C-peptide* (eds Baba, S., Kaneko, T. & Yanaihara, N.) 419–425 (Excerpta Medica, Amsterdam, 1979).
10. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **250**, 1907–1911 (1975).

BOOK REVIEWS

Robert Boyle on science and philosophy

R.E.W. Maddison

THIS volume, prepared by Dr M.A. Stewart, Senior Lecturer in Philosophy at the University of Lancaster, is an additional title in the series of Philosophical Classics published by the Manchester University Press. It is a collection of certain writings by Robert Boyle (1627–1691) “which are of primary interest to historians of philosophy and the philosophy of science, and to those who are concerned with the interaction of philosophical, scientific and religious ideas in the period of the scientific revolution”. The text of the representative writings selected for reproduction has been fully modernized so as to obviate obscurities and to give a more fluent prose for the benefit of those not acquainted with the seventeenth-century style of writing. This modernization of the original printed editions will, as the editor admits, be regarded by some as taking liberties with the text. Misprints have been corrected where necessary to restore sense to a passage; wrong references have been silently corrected; but the greatest changes have been made in punctuation. Nevertheless, a newcomer to Boyle’s writings will not find them easy reading. Robert Boyle was conscious of the deficiencies of his literary style, for the quaintness of which he was twitted in his lifetime. Samuel Johnson excused it on the ground that Boyle’s philosophical studies did not allow him time for the cultivation of style as he (Boyle) was more concerned in getting his thoughts down black on white.

Dr Stewart provides an introduction which comprises a short sketch of Boyle’s life and work, with notes on the papers in this edition and on editorial practice.

Robert Boyle became interested in natural philosophy at an early age, and accordingly instructed himself in the Aristotelean doctrine, but found that its principles were more strongly asserted than proved, and that considerable arguments could be advanced against it. His own views on the nature of matter, supported

Selected Philosophical Papers of Robert Boyle. Edited by M.A. Stewart. Pp.256. (Manchester University Press: Manchester, 1980.) £12.

by chemical and physical experiments, were briefly expounded first of all in *Certain Physiological Essays* (1661) and then in greater detail, to “serve for an introduction into the elements of the Corpuscularian philosophy”, in *The Origin of Forms and Qualities . . .* (1666) which is fully reproduced in this edition, apart from the second half of the book devoted by Boyle to “Considerations and Experiments”, which is omitted. This text outlines Boyle’s views on the nature of matter, which hypothesize the existence of simple corpuscles and their coalition to form complex ones, and led to a theory of simple substances.

This concept of matter is extended in “An Introduction to the History of Particular Qualities”, which prefixes a volume of *Tracts* (1671) wherein the first is “The Cosmical Qualities of Things”. Boyle’s “qualities” correspond to the term ‘properties’ in modern chemistry. The four elements of Aristotle — earth, fire, air and water — were abandoned together with their accompanying primary qualities — dry, hot, cold and wet — and their secondary qualities of causal derivatives. The qualities of bodies were then ascribed to various combinations of corpuscles and their local motion. He regarded matter and motion as the simplest physical principles.

With regard to Boyle’s work on the corpuscular theory of matter, Dr Stewart names three of its significant contributions to the history of ideas.

First, it led him . . . to important reflections on the nature of species, the determination of classes, and the basis of classification, which are the direct ancestor of Locke’s discussion of nominal as against real essence, and avoid some of the psychological complications in Locke’s account. Secondly, it led him into

theorizing about scientific methodology — into questions about the status of hypotheses, the role of experiment, and the criteria of a good hypothesis — about which he wrote more fully than most of his contemporaries, his views clearly crystallizing side by side with his efforts in actually promoting the corpuscularian hypothesis against its rivals; his writing as a methodologist is at least as important as his (often inconclusive) experimental work in explaining Boyle’s success as an advocate of the new philosophy. Thirdly, it led him into taking a leading part in the revival of natural theology, a movement which, despite the assaults of Hume and others a century later, continued to receive powerful support until well into the nineteenth century.

The tract entitled *Of the Imperfection of the Chemists’ Doctrine of Qualities* is a discourse included in *Experiments, Notes &c about the Mechanical Origine or Production of divers particular Qualities* (1675) and deals more concisely with matters treated more tediously in *The Sceptical Chymist* (1661). It is concerned with demolishing doctrines of Aristotelians and chemists.

An important paper on the corpuscularian hypothesis entitled “About the Excellency and Grounds of the Mechanical Hypothesis” formed an appendage to *The Excellency of Theology, compared with Natural Philosophy* (1674): it is a pious defence of theology against natural philosophy, the writing of which was perhaps stimulated by claims that natural philosophy encouraged atheism. This item provides a clear exposition of the mechanical philosophy or theory of matter in motion, and Boyle’s reasons for rejecting the Aristotelean elements with their associated forms and qualities.

On the whole it seems that Boyle’s corpuscularian hypothesis satisfies his requirements for a good and excellent hypothesis, one manuscript version of which, being notes for a projected treatise on the philosophy of science, is reproduced on p.119. Robert Boyle wrote extensively

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Robert Boyle 1627–1691

on the relation between natural science and theology, partly because he believed that study of the former is useful theologically in providing evidence for the wisdom of the Creator, and partly to combat the view held in some quarters that science tends to foster atheism. The essay entitled "A Requisite Digression concerning Those that would Exclude the Deity from intermeddling with Matter" is the fourth in *Some Considerations touching the Usefulness of Experimental Philosophy* (1663). This is one of the places in which the clock metaphor occurs, with reference to the famous astronomical clock of Strasbourg Cathedral (reproduced as the frontispiece). God not only created a mechanical world but also maintained it. Boyle did not regard theology and natural science as opposing interests; he urged a harmonious and helpful co-operation between what he probably considered as two aspects of one entity. In *The Christian Virtuoso* (1690) he wrote: "if a deep insight into nature be acquired by a man of probity and ingenuity . . . that would naturally lead him to sentiments of religion".

The whole of Section II and most of Section IV of the essay *A Free Enquiry into the Vulgarly Received Notion of Nature* (1686), have been selected for reproduction in this volume. Here, Boyle discusses the ambiguity of the term *nature*, and concludes that the vulgar notion of it is indefensible and that its use is an admission of ignorance. He discusses the main, vulgarly held views and demonstrates that they are contradicted by the facts of observation. *Ex rerum causis supremam noscere causam* was fundamental for Boyle.

In *A Discourse . . . about the Possibility of the Resurrection* (1675),

Boyle, from considerations of theology and natural philosophy, reaches the conclusion that resurrection of the body is not inconsistent with natural philosophy. Resurrection is taken as representative of a miracle, a supernatural event not capable of proof by natural philosophy, but possible through the power of God.

Finally, as a specimen of Robert Boyle's style in writing dialogue, part of *A Discourse of Things above Reason* (1681) is included, being a discussion between four characters on the question "whether, and, if at all, *how far*, we may employ our reasonings about things that are above our reason, as Christians grant some mysteries of their religion to be". Boyle regarded miracles as proof of things above reason, but believed that "reason, when assisted by revelation, may enable a man to discover far more excellencies in God, and perceive them, than he contemplated before, far greater and more distinctly". In this way a fuller comprehension of God's will and law is obtained. By philosophy Robert Boyle understood "a comprehension of all those truths or doctrines, which the natural reason of man, freed from prejudices and partiality, and assisted by learning, attention, exercise, experiments, &c, can manifestly make out, or, by necessary consequence deduce from clear and certain principles". The definite connection and interrelation of all parts of nature as created by God may be likened to, and mechanically explained as in the wonderful, intricate Strasbourg clock. □

R.E.W. Maddison is a Consulting Chemist, and sometime an Assistant Master at Wellington College, Berkshire, UK, and Leverhulme Research Fellow.

Relativity and Maxwell

W.B. Bonnor

Genesis of Relativity. By L.S. Swenson Jr. Pp.266. (Burt Franklin: New York, 1980.) \$21.

THE sub-title of this book is *Einstein in Context*, but this is an Einstein book of an unusual kind. Indeed, Einstein hardly figures in it until p.99, and then soon after he disappears again until p.148. Only the last chapter and an epilogue concentrate on Einstein's own work. The reason is that Mr Swenson uses a great deal of space to set the scene for special relativity. He is fascinated by nineteenth-century electromagnetism and gives a detailed history of it with particular reference to the period from 1860 onwards, which was dominated by Maxwell's theory. Though the connection of some of this with relativity is somewhat tenuous, it makes very interesting reading and contains much not to be found in other books on the history of the discovery of relativity.

From our fortunate viewpoint today, electromagnetism consists of four vector Maxwell equations together with the Lorentz equation of motion and various constitutive relations, and it is not easy to comprehend the turmoil the subject was in about 1880. Maxwell had put forward his equations, but insisted that there must be a medium to carry the field. Helmholtz believed in a two-fluid theory of electricity, one fluid for charge of each sign. Kelvin, along with many scientists, felt that he could not understand the theory unless he could make a mechanical model of it, and he thought in analogues of vibrating shells and plates. Moreover, the Newtonian type of theory, based on particles and the inverse square law, still had a powerful adherent in Wilhelm Weber.

A crucial test of Maxwell's theory was the transmission of electromagnetic waves, and this was demonstrated by the experiments of Hertz, a good account of which is given. An intriguing history is also given of Michelson's famous experiment to detect the ether wind, and of his bitter disappointment at the null result.

Interesting as all this is, it gives little new information on Einstein's discovery of special relativity. Here Mr Swenson's description is similar to that in other books. The marvel, of course, was how Einstein was able to ignore the tangled skein of nineteenth-century electromagnetism and pick out the very few crucial elements which were required for his theory.

There is one chapter on general relativity. This is rather disorganized as the author is inclined to ramble somewhat aimlessly through the various parts of the

Courtesy National Portrait Gallery

subject: for instance, although there is a section headed "Gravitation and Cosmology", a description of Hubble's law and cosmological models appear under another entitled "The Quest for a Unified Field Theory".

In an epilogue on Einstein there is an account of Einstein's social, political and religious views (and these appear here and there throughout the book). There is also, for anybody who is interested, a graph showing the numbers of papers Einstein

produced during the different years of his working life!

There are some infelicitous choices of words which may prevent the reader getting the meaning the author is trying to convey. For instance on p.102 one is startled to find Oliver Lodge accused of "despicable behavior": yet it transpires that his sin consisted of nothing more than turning his interests to psychical phenomena.

The book has a good index and extensive

bibliographies at the ends of the chapters. It contains practically no mathematics — but on p.124 Maxwell's equations are incorrectly given.

Those interested in the history of nineteenth-century physics and how special relativity grew out of it will enjoy this book. □

W.B. Bonnor is Professor of Mathematics at Queen Elizabeth College, University of London, UK.

Achievement in organic chemistry

Robert Ramage

Comprehensive Organic Chemistry: The Synthesis and Reactions of Organic Compounds. Overall editors Derek Barton and W. David Ollis. Six volumes. (Pergamon: Oxford and New York, 1979.) Six-volume set £687.50, \$1,375. Vol. 1. *Stereochemistry, Hydrocarbons, Halo Compounds, Oxygen Compounds*, pp.1,227 (edited by J.F. Stoddart). Vol. 2. *Nitrogen Compounds, Carboxylic Acids, Phosphorus Compounds*, pp.1,329 (edited by I.O. Sutherland). Vol. 3. *Sulphur, Selenium, Silicon, Boron, Organometallic Compounds*, pp.1,323 (edited by D. Neville Jones). Vol. 4. *Heterocyclic Compounds*, pp.1,228 (edited by P. G. Sammes). Vol. 5. *Biological Compounds*, pp.1,205 (edited by E. Haslam). Vol. 6. *Author, Formula, Subject, Reagent and Reaction Indexes*, pp.1,628 (edited by C. J. Drayton).

THIS series of volumes represents a major achievement by the editors and the many authors in producing a survey of contemporary organic chemistry which is complementary to both general textbooks and the specialized works such as Houben-Weyl (George Thieme: Stuttgart). The subject of organic chemistry has developed over the last two decades to such a degree that no one book could cover the subject to the depth required to stimulate advanced students and researchers in the discipline. Selection of topics for each volume is logical and the authors are active researchers expert in the material covered in their chapters. This situation could be expected to lead to problems of imbalanced coverage of topics, and variations in style and presentation and coverage of material, leading to overlap between the individual chapters. The editors and authors should be pleased with their efforts as deficiencies of these types are minimal within the

context of such a major work.

Volume 1 has a short introduction to stereochemistry which is excellent but deliberately stops short of dynamic stereochemistry in order to allow this important application to be introduced subsequently at appropriate points in the succeeding chapters and volumes. First reading of the stereochemistry section may at first sight be perplexing but on further consideration it really does set the scene for all that follows and shows that a great deal of thought has gone into the enterprise. The treatment of saturated hydrocarbons has an interesting structural and physical organic emphasis, whereas the articles on olefinic and acetylenic systems have a proper regard for the preparative organic chemistry of these reactive functional groups. Aromatic chemistry is dealt with in complementary ways by discussing first the development of aromaticity and then the chemical reactivity of arenes in impressive detail. The synthesis and properties of annulenes leads naturally from the preceding topics and is treated in an expert manner. Concluding the section on hydrocarbon chemistry are two interesting chapters on reactive intermediates. The remaining parts of the volume treat functional group chemistry in an orthodox manner placing more emphasis on synthesis. It is here that some questions may be asked about the balance of material in that the coverage of quinone chemistry is scant in comparison to that of other sections in Vol. 1.

The second volume covers organic nitrogen and phosphorus compounds with the chemistry of carboxylic acids interposed. The section on amines is dominated by discussion of non-aromatic types and assembles valuable information

concerning preparation, properties and reactions which is the typical format throughout. There is an adequate coverage of stereochemistry and spectroscopic properties followed by a detailed discussion of basicity in solution and the gas phase. In the section on reactions there is an introduction to strong base methodology, used widely throughout the volumes, and protection of amines is described briefly as a prelude to a later chapter on peptides. The chapter on polyfunctional amines concentrates on the reactions of a variety of structural types of relevance to pharmaceutical applications. Aromatic amines are dealt with in an unexceptional manner, followed by a fine section on hydroxylamine derivatives in which a good balance is struck between the preparative and reactive aspects including application of nitroxides to radical chemistry. Expert treatment of hydrazine chemistry is also in this volume and, although mechanisms are considered at appropriate points, the physical organic balance is maintained with a short contribution on nitrogen cations, anions, radicals and nitrenes. A good coverage of the extensive chemistry of nitro and nitroso compounds is followed by an exceptionally thorough article on imines, nitrones, nitriles and isocyanides. At this juncture Vol. 2 turns to carboxylic acid chemistry in the same logical and comprehensive manner. A modern treatment of carboxylic acids is developed into more complex systems with particular emphasis on preparative aspects. The treatment of oxo-carboxylic acids is excellent, covering both preparative and physical organic principles. The good cross-referencing between the volumes is exemplified by the short chapter on amino acids which allows continuity and prepares for the later section in Vol. 5. Two excellent chapters on esters and amides are well constructed and correlate well with preceding material. The conventional chemistry of the first-row elements is concluded with discussion of derivatives of carbon dioxide and peroxy acids. Finally, there is an excellent critical presentation of organophosphorus chemistry in which the important features of stereochemistry, preparation and reactions are identified for the non-specialist.

The last of the volumes of the first edition of *Research in British Universities, Polytechnics and Colleges*, Vol. 3 *Social Sciences*, has just been published by The British Library, Boston Spa, West Yorkshire, UK, price £10, \$22.

Volume 1 *Physical Sciences* appeared in March 1979, price £15, \$32, and Vol. 2 *Biological Sciences* in December 1979, price £10, \$22.

The second edition of Vol. 1 will be published at the end of 1980.

Volume 3 is impressive and is written by established experts in many of the fields covered. There is a thorough treatment of organosulphur chemistry (487 pages) which gives the professional chemist an excellent modern overview in which the main applications for synthesis are emphasized. The format is the same as in the preceding volumes, with little concern with detailed mechanism in the sections on synthesis and reactivity. Where applicable, stereochemical principles are discussed with additional reference to review literature. The volume abounds with interesting chemistry in fields of great current interest, for example organoselenium, tellurium to a lesser extent, and silicon chemistry. The chapter on silicon is beautifully constructed and will be most instructive to the readership for which this series is intended. The chapters on organoboron chemistry (254 pages) are indeed comprehensive and most useful. Due reference is given to existing monographs on the subject but this contribution is an intellectual achievement in its own right, correlating stereochemical and physical properties with chemical reactivity. The vast area of organometallic compounds is condensed into 383 pages with the emphasis being placed on transition metal chemistry where a wide sweep of this active area is discussed concisely.

The task of constructing a single volume on heterocyclic compounds is a daunting one which has largely been met in Vol. 4. The decision was obviously made to concentrate on aromatic and conjugated systems, placing strong emphasis on the preparation and reactions of the main ring-systems. Even with such constraints, the selection and organization of material is of great importance so that the fundamentals, once learned, can be applied to the many heterocyclic systems omitted from this work by necessity. With this in mind, this contribution to the series successfully surveys the field by devoting 604 pages to nitrogen heterocycles, 182 pages to oxygen heterocycles, 172 pages to sulphur and related systems, and 269 pages to mixed heteroatom systems. As expected in such a multi-author work, the presentation varies but overall is of high quality. The section on nitrogen systems is detailed and useful to non-specialists. Reference is given to naturally occurring systems throughout as examples of structural types. The pyrrole, porphyrin and indole chapters give an easy entry into these specialized themes. Treatment of the biologically important purines is expert and thorough, and will serve as a useful introduction to the chemist wishing to enter the field. The section on oxygen heterocycles gives a concise account of five- and six-membered systems, and it is interesting to compare the presentation of this section with the later chapters on organosulphur systems which contain much useful chemistry for the reader. Finally, the treatment of mixed heterocyclic systems will prove invaluable

to those entering pharmaceutical chemistry. A minor criticism could be made that with the enormous volume of chemistry to be compressed into a single volume the discussion occasionally degenerates to a cataloguing of results.

The editor of Vol. 5 had the slightly easier task of covering biological chemistry in one volume. To accomplish this meant careful selection of chapter topics and materials within individual chapters with corresponding unavoidable omissions. Again, an impressive list of experts has performed the task exceptionally well and the result of their efforts will be invaluable to specialists as well as chemists lacking biochemical training. The approach to the subject is a critical one depending heavily on experimental results with important references to vital synthetic transformations. Coverage of primary and secondary metabolites is well balanced, with an important contribution on the mechanistic aspects of enzyme catalysis. Altogether this volume makes fascinating reading.

The last volume serves to endorse the comprehensive nature of the series as it contains an impressive range of indexes which enable easy retrieval of information and cross-referencing from the previous five volumes. Direct access to specific compounds may be gained from the formula index which contains around 20,000 formulae. The subject index refers both to classes of organic compounds and reaction types, and to important specific compounds. Experienced researchers will find the excellent author index a useful and

rapid entry into the series. The reaction index has entries to material covered in Vols 1-5 together with useful supplementary lists of review articles. These entries consist of reaction types and named reactions, in addition to a listing of reagents which leads the reader to the extensive reagent index of over 25,000 compounds. This index not only serves a primary function but in addition provides access to supporting literature references.

It would be unrealistic to expect the content of the volumes to be complete in terms of the present knowledge of the subject and one has to accept the selection of material by the contributors whose judgement is of a high standard. Fortunately there are few omissions and even these can reflect the predilections of the reader. There are relatively few errors, mainly in structures, for such a large multi-author work, and these will no doubt be remedied by the publishers. I found the books to be very enjoyable and informative to read as well as extremely useful for reference in research and teaching exercises in a wide variety of topics over a realistic period of time for assessment. Thus the overall impression of *Comprehensive Organic Chemistry* is that the series is indeed comprehensive and will be an essential purchase for chemical libraries. Sadly the cost will limit the number of private purchasers of this important work.

Robert Ramage is Professor of Organic Chemistry at the University of Manchester Institute of Science and Technology, UK.

Indulging in developmental biology

D. R. Garrod

Introductory Concepts in Developmental Biology. By A. Monroy and A. A. Moscona. Pp.252. (University of Chicago Press: Chicago and London, 1980.) \$16, £9.60.

THIS book claims to be (I quote from the preface) "an up-to-date introduction for beginners" and "a guidebook for graduate students and teachers to some issues, answers and challenges in developmental biology", as well as a "balanced sampling of facts, concepts and projections". As a teacher of developmental biology at elementary and advanced level for nearly a decade, my interest was aroused and I proceeded with anticipation. I was soon disappointed: the claim is too ambitious.

Chapter 1 is a brief descriptive embryology similar to, and largely derived from, previous books on the subject.

Thereafter, the book abandons any pretence at being a balanced, introductory guidebook and divides rather neatly into two sections, early development (Chapters 2 and 6 dealing with oögenesis, fertilization, cleavage and gene expression), and morphogenesis and cell aggregation (Chapters 7 and 8 respectively). No attempt is made to link these two aspects and one might be forgiven for feeling that this arrangement (and perhaps the entire book) serves no other purpose than to indulge the research interest of the authors. This is not necessarily a bad thing, but I cannot recommend the book as a students' introduction to developmental biology because many important aspects of the subject, such as organogenesis, pattern formation and differentiation, are hardly mentioned.

In some respects the book succeeds better as a review for advanced students. With the proviso that early development is not my field, I found the treatment of it comprehensive and informative. The classical work is fully outlined and many more recent developments are presented. It seems like good solid stuff — not particularly exciting, but certainly adequate.

I had more difficulty with the section on

morphogenesis and cell aggregation, perhaps because it is my field. My main objection is that it is not 'balanced'. There are many types of morphogenetic movement, yet only one class of them, long-distance cell migrations of, for example, primordial germ cells and neural crest, are considered here. Other types of morphogenetic movement — the majority in fact — such as the bending of cell sheets and the formation of tubules are not considered. The final chapter, about cell aggregation, is really little more than a review of the work of one of the authors (21 out of 28 figures in this chapter are from his own work). I would point out that cell

aggregation is not an aspect of normal development and that the significance of much of this work is questionable. Surely this is not the way to present beginners with a balanced view of developmental biology.

The figures in the book are generally of a very high standard, the bibliography is good and the index useful, though it might have been better to have separated author and subject indexes.

All in all this is a poor book, and one rather unsuitable for beginners. □

D. R. Garrod is a Lecturer in Medical Oncology at the University of Southampton, UK.

Exciting dilution analysis

Elizabeth Simpson

Limiting Dilution Analysis of Cells in the Immune System. By I. Lefkovits and H. Waldmann. Pp.262. (Cambridge University Press: Cambridge, UK, 1979.) £15, \$36.

LIMITING dilution analysis is a new and very powerful way of examining cell-cell interactions in the immune response. Subpopulations of lymphocytes defined with respect to different functions and by surface markers exist, and questions of interactions between them can only be partially analysed *in vivo* or *in vitro* in bulk cultures and mixing experiments. Limiting dilution analysis allows more precise and quantitative questions to be asked. This book is an extensive treatise on the rationale of this approach. It includes both a detailed mathematical analysis and methodological descriptions so that the newcomer could set up limiting dilution experiments using the book and the examples in it as a text.

The opening chapters on the immunology of a single cell (Chapter 1) and manipulation of lymphocytes (Chapter 2) describe the immunological background clearly and well. There are minor lapses such as failure to specify whether bicarbonate- or phosphate-buffered balanced salt solutions (BSS) are used in the preculture manipulations of lymphocytes (e.g. in nylon column separations of T cells) or whether 37°C incubations in BSS

are carried out in a CO₂ incubator. Also, test tubes used in some manipulations are given a 'house' name only, e.g. Wasserman tube (p.22) or rhesus tube (p.27), and further identifying specifications are not given.

Chapter 3, 'Methods of Determining B Cell Responses', is detailed and gives a comprehensive description of methods that can be adapted for use on single clones including plaquing, isoelectric focussing and radioimmunoassay.

Chapter 4, on Poisson distribution, includes a discussion of single-hit, multi-hit and multi-target events, vital for any analysis of immunological responses many of which certainly involve the interaction between two or more cell types. This is illustrated by experimental examples of titration of B precursor cells and T helper cells in Chapter 5. Further experimental data are to illustrate clone size estimation (Chapter 6). Chapter 8 describes results on the analysis of B cell responses, and Chapter 9 results on the analysis of T helper cells. These two chapters contain details of experiments performed mainly by the authors during investigations of questions they have answered uniquely by using the limiting dilution analysis.

The weakest and least developed chapter is that on analysis of other T cell functions (Chapter 10) including cytotoxic T cells. Results of frequency estimations of alloreactive cytotoxic precursors are described but from the experimental details given it would not be possible to do such experiments, and this is in contrast to the sort of experimental detail used to illustrate B cell and T helper cell function. Furthermore, no consideration is given to the presence of T helper cells for cytotoxic responses and this certainly does affect the analysis.

Nevertheless this book is an excellent one: it is timely, comprehensive, well written and transmits the authors' excitement at using limiting dilution analysis to approach questions of lymphocyte function at the single-cell level. □

Elizabeth Simpson is Senior Research Scientist at the Transplantation Biology Unit, MRC Clinical Research Centre, Harrow, UK.

Hard science in renewable energy

Chris Hope

Renewable Energy. By B. Sørensen. Pp.583. (Academic: London and New York, 1979.) £20, \$46.

I AM always wary of a book with a title so general that it implies a great breadth of coverage. All too often the reality is a book full of the cream skimmed off the surface of a subject — tasty in mouthfuls but totally unsatisfactory as a main course. When the subject is also of intense topical interest, the suspicion that the glossy covers contain nothing that will be worth recalling next year becomes almost a certainty. This book is a reminder that the improbable does happen, for it contains a deep treatment of aspects of renewable energy that I suspect will be of lasting use.

The three chapters (460 pages) concerning the origin, diverse manifestations and means of harnessing the renewable energy flows form a comprehensive treatment of the methods and principles needed to understand what it is reasonable to expect from these sources. Techniques employed include meteorology, geology and chemistry, but with a preponderance of the various branches of physics, and it is these chapters on the hard science of the 'renewables' that will make this an enduring basic text. Succeeding chapters treat energy storage and energy supply systems in a similarly technical manner.

This central scientific core is preceded by a short scene-setting chapter that comes close to sensationalism in its comparison of Man's energy usage over the last half-million years with the enormously greater amounts of energy involved in keeping our planet in its allotted place in the heavens (approximately 8×10^{33} J, if you didn't know). The book concludes with a subjective account of socio-economic assessment. This is the only chapter in which the author's strong personal views lead him in places to write as an advocate of renewable energy rather than an impartial observer.

Of course this book will not appeal to as wide an audience as a skimmed-cream book would have done. There are no descriptions of actual devices 'as constructed by the author', no colour photographs of windmills against a blue sky, just a host of line drawings, a good deal of fairly advanced mathematics and a thorough treatment of one aspect of a small but important topic typified by the inclusion of 500 references and a 1000-item index.

I recommend this book, especially to anyone running, or thinking of setting up, a course on the physics of energy. □

Chris Hope is a member of the Energy Research Group at the University of Cambridge, UK.

In the review of *Image Analysis, Enhancement and Interpretation*, edited by D. L. Misell (*Nature* 282, 885; 1979), the prices were incorrectly quoted, thus making the final criticisms of the reviewer unfounded. The book is available in paperback, price \$31.75, Dfl. 65, or in hardback as Vol.7 of the series *Practical Methods in Electron Microscopy*, price \$66, Dfl. 135.

BOOKS RECEIVED

Earth Sciences

LENIHAN, J. and FLETCHER, W.W. (ed.) *The Biological Environment. Environment and Man Volume 9*. Pp. iv + 164. ISBN-0-12-443509-2. (New York and San Francisco: Academic, 1979.) £17.50.

LOWE, J.J., GRAY, J.M. and ROBINSON, J.E. (ed.) *Studies in the Lateglacial of North-West Europe. Including Papers presented at a Symposium of the Quaternary Research Association held at University College London, January, 1979*. Pp. xii + 203. ISBN-0-08-024001-1. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon, 1980.) US \$38.00, £17.00.

McROSTIE, C.N. (ed.) *Global Resources: Perspectives and Alternatives*. XIV Nobel Conference. Pp. x + 97. ISBN-3-540-90397-6. (New York, Heidelberg, Berlin: Springer, 1979.)

SHARMA, G. D. *The Alaskan Shelf; Hydrographic, Sedimentary, and Geochemical Environment*. Pp. xiii + 498. ISBN-3-540-90397-6. (New York, Heidelberg, Berlin: Springer-V, 1979.)

SITTIG, M. (ed.) *Geophysical and Geochemical Techniques for Exploration of Hydrocarbons and Minerals*. Pp. ix + 229. ISBN-0-8155-0782-8. (New Jersey: Noyes Data Corporation, 1980.) \$40.00.

SMITH, R.E. *Lower Devonian (Lochkovian) Biostratigraphy and Brachiopod Faunas, Canadian Arctic Islands*. Geological Survey Bulletin 308. Pp. x + 155. ISBN-0-660-10221-8. (Quebec: Canadian Government Publishing Centre, 1980.) Canada \$5.00 other countries \$6.00.

TOKSOZ, M.N., UYEDA, S. and FRANCHETEAU, J. (ed.) *Oceanic Ridges and Arcs; Geodynamic Processes. Developments in Geotectonics 14*. Pp. xvi + 538. ISBN-0-444-41839-3. (Amsterdam, Oxford, New York: Elsevier Scientific, 1980.) US \$29.25, Dfl. 60.00.

UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE. *Selected Water. Problems in Islands and Coastal Areas. With special regard to Desalination and Groundwater. Proceedings of a seminar organized by the Committee on Water Problems of the UNECE, Malta, June, 1978*. Pp. xxv + 501. ISBN-0-08-024447-5. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon, 1979.) £34.00, \$68.00.

WALKER, D. and GUPPY, J.C. (ed.) *Biology and Quaternary Environments. Based on the Symposium on Biological Problems in the Reconstruction of Quaternary Terrestrial Environments, held in Canberra, February, 1978*. vii + 264. ISBN-0-85847-050-0. (Canberra: Australian Academy of Science, 1978.) A\$11.00 flexi.

WEST, R.G. *The pre-glacial Pleistocene of the Norfolk and Suffolk coasts*. Pp. xi + 239. ISBN-0-521-21962-0. (Cambridge: Cambridge University Press, 1980.) £40.00.

Biology

AKINS, F.R., MACE, S.M., HUBBARD, J.W. and AKINS, D.L. *Behavioral Development of Nonhuman Primates. An Abstracted Bibliography*. IFI Data Base Library. Pp. ix + 304. ISBN-0-306-65189-0. (New York, Washington, London: IFI/Plenum, 1980.) \$75.00.

ARBER, W. et al. (ed.) *Current Topics in Microbiology and Immunology* volume 88. Pp. iii + 142. ISBN-3-540-09415-6. (Berlin, Heidelberg, New York: Springer-V, 1979.) DM 64 approx. US \$35.90.

ARBER, W. et al. (ed.) *Current Topics in Microbiology and Immunology* volume 87. Pp. iii + 172. ISBN-3-540-09433-4. (Berlin, Heidelberg, New York: Springer-V, 1979.) DM 68 approx. US \$38.10.

ARMSTRONG, M. A. *Basic Topology*. Pp. xii + 251. ISBN-0-07-084090-3. (Maidenhead: McGraw-Hill, 1979.) £7.25 flexi.

AVERS, C. J. *Genetics*. Pp. xi + 659. ISBN-0-442-26233-7. (New York, Cincinnati, Toronto, London, Melbourne: D. Van Nostrand and Company, 1980.) \$21.95.

BARAM, P., BATTISTO, J. R. and PIERCE, C. W. (ed.) *Immunologic Tolerance and Macrophage Function. Developments in Immunology Volume 4. Proceedings of the 7th Annual Mid-West Autumn Immunology Conference, Dearborn, Michigan, U.S.A., November 1978*. Pp. xiii + 266. ISBN-0-444-00316-9. (New York, Amsterdam, Oxford: Elsevier/North-Holland, 1979.) \$40.00.

BARRINGTON, E. J. W. (ed.) *Hormones and Evolution Volume 2*. Pp. xxv + 989. ISBN-0-12-079402-0. (New York, San Francisco, London: Academic, 1979.) £30.00 \$69.00.

BARRINGTON, E. J. W. (ed.) *Hormones and Evolution Volume 1*. Pp. xxv + 491. ISBN-0-12-079401-2. (New York, San Francisco, London: Academic, 1979.) £30.00 \$69.00.

BEGLEY, D. J., FIRTH, J. A. and HOULT, J. R. S. *Human Reproduction and Developmental Biology*. Pp. x + 250. ISBN-0-333-23423-5 hardback ISBN-0-333-2324-3 paperback. (London and Basingstoke: T. Macmillan P. Ltd. 1980.) £20.00 hardback £9.95 pbk.

BEHRESEMEYER, A. K. and HILL, A. P. (ed.) *Fossils in the Making. Vertebrate Taphonomy and Paleocology. Prehistoric Archeology and Ecology. Series. Pp. xii + 338. ISBN-0-226-04168-9. (Chicago and London: The University of Chicago Press, 1980.) \$7.00.*

EISENBERG, J. F. (ed.) *Vertebrate Ecology in the Northern Neotropics. Results from a Team research Effort sponsored by the National Zoological Park, Smithsonian Institution, March 1973 to August 1978 and a Workshop held at the National Zoological Park on December 14, 1978*. Pp. 271. ISBN-0-87474-410-5 hardback ISBN-0-87474-409-1 pbk. (Washington, D.C.: Smithsonian Institution Press, 1980.) \$17.50 hardback \$8.95 pbk.

BOURNE, G. H. and DANIELLI, J. F. (ed.) *International Review of Cytology* volume 63. Pp. vii + 410. ISBN-0-12-364463-1. (New York, London, Toronto, Sydney, San Francisco: Academic Press, 1980.) \$30.00.

BAUMEL, J. J. et al. (ed.) *Nomina Anatomica Avium. An annotated Anatomical Dictionary of Birds*. Pp. xxv + 637. ISBN-0-12-08150-3. (London, New York, Toronto, Sydney, San Francisco: Academic Press, 1979.) £28.00 \$64.50.

CANTOR, C. R. and SCHIMMEL, P. R. *Biophysical Chemistry part 1. The Conformation of Biological Macromolecules*. Pp. xxvii + 341 + Appendices ISBN-0-7167-1042-0 hardback ISBN-0-7167-1188-5 pbk. (San Francisco: Freeman, 1980.) \$30.00 hardback \$16.95 pbk.

CLOUDSLEY-THOMPSON, J. L. *Tooth and Claw. Defensive Strategies in the Animal World*. Pp. 252. ISBN-0-460-04360-9. (London: J. M. Dent & Sons Ltd., 1980.)

COHEN, M. P. and FOA, P. P. (ed.) *Special Topics in Endocrinology and Metabolism Volume 1*. Pp. viii + 144. ISBN-0-8451-0700-3. (New York: Alan R. Liss, Inc. 1979.) np. Available in UK, Europe and Middle East through European Book Service.

COHN, W. E. (ed.) *Progress in Nucleic Acid Research Molecular Biology. Volume 23*. Pp. xv + 299. ISBN-0-12-540023-3. (New York, London, Toronto, Sydney, San Francisco: Academic Press, 1980.) \$31.00.

COUGHLAN, M. P. (ed.) *Molybdenum and Molybdenum-Containing Enzymes*. Pp. xii + 577. ISBN-0-08-024398-3. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon P, 1979.) \$80.00 £36.00.

CREUTZFELDT, O., SCHEICH, H. and SCHREINER, Chr. (ed.) *Hearing Mechanisms and Speech. EBBS-Workshop, Göttingen, April 26-28, 1979. Experimental Brain Research Supplementum 2*. Pp. xxiii + 413. ISBN-3-540-09655-8. (Berlin, Heidelberg, New York: Springer-V., 1979.) DM 42 approx. US \$23.10 flexi.

DE BRABANDER, M., MARREL, M. and DE RIDDER, L. (edited by.) *Cell Movement and Neoplasia. Proceedings of the Annual Meeting of the Cell Tissue and Organ Culture Study Group, held at the Janssen Research Foundation, Beerse, Belgium, May, 1979*. Pp. vii + 174. ISBN-0-08-025534-5. (Oxford, Pergamon, 1980.) £28.00 £13.50.

DORMER, K. J. *Fundamental tissue geometry for biologists*. Pp. vi + 149. ISBN-0-521-22326-1. (Cambridge, London, New York, New Rochelle, Melbourne, Sydney: Cambridge University Press, 1980.) £15.00.

EBBESON, S. O. E. (ed.) *Comparative Neurology of the Telencephalon*. Pp. xxi + 506. ISBN-0-306-40237-8. (New York and London: Plenum, 1980.) \$45.00.

ELLENBERG, H., ESSER, K., KUBITZKI, K., SCHNEPP, E. and ZIEGLER, H. (ed.) *Progress in Botany. Morphology, Physiology, Genetics, Taxonomy, Geobotany. Volume 41*. Pp. xi + 356. ISBN-3-540-09769-4. (Berlin, Heidelberg, New York: Springer, 1979.) DM 119 approx. US \$66.70.

FINN, C. A. (ed.) *Oxford Reviews of Reproductive Biology. Volume 1, 1979*. (Oxford Science Publications). Pp. viii + 485. ISBN-0-19-857534-3. (Oxford: Clarendon Press, Oxford University Press, 1980.) £30.00.

FLIEGG, J. (Consultant editor.) *The British Ornithologists' Guide to Bird Life*. Pp. xi + 318. ISBN-0-7137-0996-0. (Poole: Blandford Press, 1980.) £10.95.

GIMENEZ, M. and SAUNIER, C. (ed.) *Muscular Lung Exercise in Chronic Lung Disease. Entretiens de Physio-Pathologie Respiratoire Nancy 11e Série*. Pp. v + 436. ISBN-0-08-024930-2. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon P, 1980.) £20.00 \$45.00.

GOY, R. W. and McEWEN, B. S. *Sexual Differentiation of the Brain. Based on a Work Session of the Neurosciences Research Program*. Pp. ix + 223. ISBN-0-262-07077-4. (Cambridge, Mass. and London: The MIT, 1980.) \$17.50.

GRIDGMAN, N. T. *Biological Sciences at the National Research Council of Canada: The Early Years to 1952*. Pp. xxi + 153. ISBN-0-88920-082-3. (Ontario: Wilfrid Laurier University Press, 1979.) \$7.50.

GROSCH, D. S. and HOPWOOD, L. E. *Biological Effects of Radiations. Second edition*. Pp. xv + 338. ISBN-0-12-304150-3. (New York, San Francisco, London: Academic P, 1979.) \$27.50.

HARRISON, G. H. *Roger Tory Peterson's Dozen Birding Hot Spots*. Pp. 288. ISBN-0-671-22329-1 hardback ISBN-0-671-25405-7 paperback. (New York: Simon and Schuster, 1980.) \$6.95 paperback.

HARRISON, R. J. *Marine Mammals 2nd Edition*. HARRISON, R. J. and KING, J. E. *Marine Mammals, 2nd edition*. Pp. 192. ISBN-0-09-140931-4. (London, Melbourne, Sydney, Auckland, Johannesburg: Hutchinson, 1980.) £23.95 flexi.

HECHT, M. K., STERE, W. C. and WALLACE, B. *Evolutionary Biology volume 12*. Pp. xii + 388. ISBN-0-306-40267-X. (New York and London: Plenum, 1980.) \$32.50.

HENDERSON, B. E. (ed.) *Second Symposium on Epidemiology and Cancer Registries in the Pacific Basin. National Cancer Institute Monograph 53*. Pp. vi + 205. (Washington: U.K.S. Government Printing Office, 1979.) \$7.50.

HERTMAN, I., SHAFFERMAN, A., COHEN, A. and SMITH, S. R. (ed.) *Extrachromosomal Inheritance in Bacteria. Contributions to Microbiology and Immunology Volume 6*. Pp. xiv + 238. ISBN-3-8055-2943-0. (Basel, München, Paris, London, New York, Sydney: S. Karger, 1979.) Sfr. 106.00 DM 127.00 approx. US \$63.50 flexi.

HOBBSON, J. A. and BRAZIER, M. A. B. (ed.) *The Reticular Formation Revisited: Specifying Function for a Nonspecific System. International Brain Research Organization Monograph Series volume 6*. Pp. xv + 552. ISBN-0-89004-379-5. (New York: Raven P, 1980.) £9.50.

HOUCK, J. C. (ed.) *Chemical Messengers of the Inflammatory Process. Handbook of Inflammation, Volume 1*. Pp. xvii + 422. ISBN-0-444-80121-9. (Amsterdam and New York: Elsevier/North-Holland Biomedical Press, 1979.) US \$75.00 Dfl. 164.00.

HSIE, A. W., O'NEILL, J. P. and McELHENY, V. K. (ed.) *Mammalian Cell Mutagenesis: The Naturalization of Test Systems. Banbury Report 2*. Pp. xiv + 504. ISBN-0-87969-4. (Cold Spring Harbor: Cold Spring Harbor Laboratory, 1979.) np.

HUBEL, D. H. et al. *The Brain. A Scientific American Book*. Pp. viii + 149. ISBN-0-7167-1150-8 board ISBN-0-7167-1151-6 pbk. (Oxford: W. H. Freeman and Company Limited, 1980.) £9.10 board £3.30 pbk.

JAMES, V. H. T. and PASQUALINI, J. R. (ed.) *Hormonal Steroids. Proceedings of the Fifth International Congress on Hormonal Steroids, New Delhi, October/November 1978*. Pp. xviii + 1068. (Oxford, New York, Toronto, Paris, Frankfurt, Sydney: Pergamon P, 1979.) \$120.00 £60.00.

JOHNSTON, F. E., ROCHE, A. F. and SUSANNE, C. (ed.) *Human Physical Growth and Maturation; Methodologies and Factors. NATO Advanced Study Series volume 30*. Pp. xii + 364. ISBN-0-306-40420-6. (New York and London: Plenum Press, 1980.) \$42.50.

KANDEL, E. R., KRASNE, F. B., STRMwasser, F. and TRUMAN, J. W. *Cellular Mechanisms in the selection and modulation of behaviour. Neurosciences Research Program Bulletin Volume 17 No. 4*. vi + 187. ISSN-0028-3967. (Cambridge, Massachusetts: MIT, 1979.)

KROG, H., ØSTHAGEN, H. and TONSBERG, T. *Lavlor. Norske busk-og bladlav*. Pp. 312. ISBN-82-00-01907-1 ISBN-82-00-01988-8 (with supplement in English). (New York: Columbia University Press; London: Global Book Resources, Ltd., 1980.) Kr. 189.00 Kr. 209.00 m/eng. Bestemmelsesnøkkel.

KUMAR S. (ed.) *Biochemistry of Brain*. Pp. vii + 625. ISBN-0-08-012345-6. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$100.00 £50.00.

LARSEN, K. and HOLM-NIELSEN, L. B. (ed.) *tropical Botany. Proceedings of a Symposium held at University of Aarhus, August, 1978*. xi + 453. ISBN-0-12-437350-X. (London, New York, San Francisco: Academic, 1979.) \$66.50 £22.80.

LEE, C. P., SCHATZ, G. and ERNST, L. (ed.) *Membrane Bioenergetics. Based on the International Workshop held at Granbrook, Michigan, 5-7 July, 1979*. Pp. xxiv + 604. ISBN-0-201-03999-0 pbk. (London, Amsterdam, Ontario, Sydney, Tokyo: Addison-Wesley Publishing Company, 1980.) US \$25.50 flexi. Also available as hardback.

LEVIN, H. and ADDIS, A. B. *The Eye-Voice Span*. Pp. viii + 165. (Cambridge, Mass. and London: MIT, 1980.) £9.30.

LITTLE, R. J. and JONES, C. E. *A Dictionary of Botany*. Pp. ix + 400. ISBN-0-442-24169-0. (Wokingham: Vn Nostrand reinhold company Ltd., 1980.) £13.90.

LOFFLER, H. (ed.) *Neusiedlersee: The Limnology of a shallow lake in Central Europe. Monographiae Biologicae volume 37*. Pp. x + 561. ISBN-0-6193-089-8. (The Hague, Boston, London: Dr. W. Junk bv., 1980.) Dutch Guilders 195 US \$102.65.

MAFAMOROSCH, K. and HIRUMI, H. (ed.) *Practical Tissue Culture Applications. Proceedings of a Conference held at the International Laboratory for Research on Animal Diseases in Nairobi, Kenya August, 1978*. Pp. xiii + 426. ISBN-0-12-470285-6. (New York, London, Toronto, Sydney, San Francisco: Academic, 1979.) \$29.50.

MELENDE, A. *Les Manipulations Génétiques*. Pp. 332. ISBN-2-02-005437-X. (Paris: Editions du Seuil, 1980.) flexi.

MARCHALONIS, J. J. and COHEN, N. *Contemporary Topics in Immunobiology. Volume 9. Self/Non-self Discrimination*. Pp. xvi + 293. ISBN-0-306-40263-7. (New York and London: Plenum P, 1980.) \$29.50.

MELNICK, M. and JORGENSEN, R. (ed.) *Developmental Aspects of Craniofacial Dysmorphology. The National Foundation-March of Dimes Birth Defects: Original Article Series, Volume xv, Number 8, 1979*. Pp. xi + 118. ISBN-0-8451-1033-0 (616' 043) 79-2487. (New York: Alan R. Liss, Inc., 1979.) \$16.00. This title is available in Europe, the United Kingdom and the Middle East exclusively from: European book Service, Flevolaan 36-38 P.O.B. 124 1380 AC Weesp, Holland. Dfl. 48.00.

ANNOUNCEMENTS

Awards

The Council of the British Institute of Radiology have made the following awards: The Barclay Medal to **Dr R. H. Mole** (MRC Radiobiological Unit, Harwell, UK). The Barclay Prize to **Dr H. B. Meire** (Clinical Research Centre, Harrow, UK). The Röntgen Prize to **Dr S. Dische** (Mount Vernon Hospital, Middlesex, UK).

Professor R. W. Baldwin Cancer Research Campaign Laboratories at the University of Nottingham UK) has been awarded the Rabbi Shai Shacknai Memorial Prize at the Hebrew University in Jerusalem.

The 1979 ICI Diagnostic Techniques Prize of £1,000 has been awarded to **Dr M. L. Little** and **Mr R. Glanville** of the Central Electricity Generating Board (UK).

The Marconi International Fellowship invites nominations for the Seventh Marconi Fellowship Award to be presented in 1981. Nominations close the 1 October 1980. For nominations in 1980, candidates will be judged on the basis of qualifications in either: (1) Contributions to the science of technology of communications or to applications thereof, for betterment of the lives of children. (2) Contributions to fundamental knowledge or practical applications of systems that contribute to the betterment of the human condition through communications by non-verbal means. Send nominations to: Dr Walter Orr Roberts, The Marconi Fellowship Council, Aspen Institute for Humanistic Studies, 1229 University Avenue Boulder, Colorado 80302.

Twenty Harkness Fellowships tenable for between 12 and 21 months are offered each year. The award includes return fares to the US, living and family allowances, travel in America, tuition and research expenses, a book and equipment allowance and health insurance. Candidacy is open to men and women in any profession or field of study, who are citizens of the UK and have received both secondary schooling and further education (or equivalent professional experience) wholly or mainly in the UK. Candidates must be between 21 or 30 years of age on 1 September 1981, unless qualified in medicine or employed in the Civil Service or the media, in which cases the upper age limit is 33. For application forms (available only to individual candidates) send a s.a.e. not less than 10 inches by 7 inches carrying 22p postage, to Harkness Fellowships (UK), Harkness House, 38 Upper Brook Street, London W1, UK.

The Council of the Eugenics Society is prepared to support research in those areas for which the late Dr Marie Carmichael Stopes is best remembered: fertility control; differential fertility; eugenic aspects of reproduction and population; eugenic aspects of sex education and sexual behaviour and the impact of all these and related matters on the welfare of women and of the community, with particular emphasis (in keeping with the biosocial outlook of the Society) on interdisciplinary research. For further information contact: The Eugenics Society, 69 Eccleston Square, London SW1, UK.

Appointments

The Royal Society have elected the following foreign members: **Professor Hannes Olof Gösta Alfvén** (Royal Institute of Technology, Sweden); **Professor Philip Warren Anderson** (Bell Laboratories, USA).

The National Academy of Sciences have elected the following foreign associates: **Ignacio Bernal y Garcia Pimentel**, (National University of Mexico); **Jacques-Emile Blamont**, (CNRS, France); **John G. Bolton**, (CSIRO, Australia); **Paul Erdős**, (Hungarian Academy of Sciences); **Roger Erdős**, (Hungarian Academy of Sciences); **Roger Gautheret**, (L'Institut de France); **John B. Gurdon**, (MRC, UK); **Jean-Marie Pierre Lehn** (Université Louis Pasteur, France); **Peter Reichard**, (Medical Nobel Institute, Sweden); **Hans H. Ussing**, (University of Copenhagen, Denmark); **Leon Van Hove**, (European Organization for Nuclear Research, Switzerland); **Torsten N. Wiesel** (Sweden); **Maurice V. Wilkes**, (University of Cambridge, UK).

The Council of the Zoological Society of London have appointed **Dr. John P. Hearn** as Director of Science and Director of the Institute of Zoology from 1 August 1980, on the retirement of **Dr L. G. Goodwin**.

Meetings

10-12 June, **Developments in Power-System Protection**, London (IEE, Savoy Place, London WC2, UK).

16-18 June, **Mass Spectrometry in Biochemistry, Medicine and Environmental Research**, Milan (Dr A. Frigerio, Italian Group for Mass Spectrometry in Biochemistry and Medicine, Via Eritrea, 62, 20157 Milan, Italy).

18-19 June **NMR Spectroscopy in Solids**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

14-17 July, **Metallo-organics in Organic Synthesis**, Swansea (Dr John F. Gibson, The Chemical Society, Burlington House, London W1, UK).

14-17 July, **Biological Reactive Intermediates Chemical Mechanisms and Biological Effects**, Guildford (Dr G. G. Gibson, Dept of Biochemistry, University of Surrey, Guildford, Surrey, UK).

21-25 July, **Stereoelectronics Effects in Organic Chemistry**, St Andrews (Dr F. F. Gibson, The Chemical Society, Burlington House, London W1, UK).

25 July, **HPLC of Drug Metabolites**, Guildford (Dr E. Reid, Wolfson Bioanalytical Unit, IIEHS, University of Surrey, Guildford, UK).

2-9 August, **The Social Context of Energy**, Harlech (Miss J. Miller, Dept of Liberal Studies in Science, University of Manchester, Manchester, UK).

5-6 September, **Symposium Mammographicum '80**, London (The British Institute of Radiology, 32 Welbeck St, London W1, UK).

14-28 September, **Advanced Techniques in Immunological and Biochemical Approaches to Hemoparasitic Research**, Nairobi (Dr R. O. Williams, ILRAD, PO Box 30709, Nairobi, Kenya).

16-19 September, **Eurotunnel '80**, Basle (The Secretary, The Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

18 September, **Some Aspects of the Biological Basis and the Clinical Effects of Different Radiotherapeutic Practices**, Oxford (Dr C. H. Paine, Dept of Radiotherapy and Oncology, Churchill Hospital, Headington, Oxford, UK).

19 September, **Directions in Nuclear Engineering Research**, Cambridge (The Institution of Nuclear Engineers, Allan House, 1 Penderley Road, London SE6, UK).

23-25 September, **On-line Surveillance and Monitoring of Plant Reliability**, London (Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1 UK).

25-26 September, **Hard-facing materials in Nuclear Power Plants**, Avignon (SFEN, 48 rue de la Procession, F75724 Paris Cedex 15, France).

29 September-2 October, **Forestry Energy Conference**, Jonkoping (Howard Phillips Management, 7 Black ans Lane, Hadlow, Tonbridge, Kent, UK).

1 October-31 August 1981, **Training Course on Selected Topics of Modern Biology**, Szeged (Dr István Raskó Biological Research Center, Hungarian Academy of Sciences, PO Box 521, H-6701 Szeged, Hungary).

9 October, **Light Scattering Techniques**, London (Mr D. Irish, c/o Pye Unicam Ltd, York St, Cambridge, UK).

Essential information at your fingertips Every month!

The British Journal of Clinical Pharmacology is the leading authoritative journal presenting results of clinical pharmacological research.

Published monthly on behalf of the British Pharmacological Society the journal:

- ★ Bridges the gap between medical professions, clinical research and the Pharmaceutical industry.
- ★ Contains papers and reports on all aspects of drug action in man, in both health and disease.
- ★ Prints letters and short communications which allow immediate discussion of urgent observations.

Subscribers to British Journal of Clinical Pharmacology receive, free of charge, the proceedings of selected symposia on new methods, new drugs, and new approaches to treatment. Some past symposia have been on narcotic antagonists and antagonist analgesics, labetalol, clobazam, mianserin, temazepam and the relationship between angina pectoris and hypertension.

Why not join the growing number of clinical pharmacologists, clinicians and others who rely on the British Journal of Clinical Pharmacology as their information medium.

For a sample copy write to: Frances Roach, Scientific & Medical Division, Macmillan Publishers Ltd., Houndmills, Basingstoke, Hampshire, RG21 2XS.

13-14 October, **New Technologies in Health Care**, Brussels (R. S. First Inc, 19a Ave Marnix, 1050 Brussels, Belgium).

13-17 October, **2nd International Conference of Scientific Editors**, Amsterdam (Helena Tombal, Elsevier Scientific Publishing Co, PO Box 330, 1000 AH Amsterdam, The Netherlands).

14-17 October, **Water Chemistry of Nuclear Reactor Systems**, Bournemouth (British Nuclear Energy Society, 1-7 Great George St, London SW1, UK).

15-16 October, **Calcitonin 1980**, Milan (Dr M. L. Pecile, Dept of Pharmacology, School of Medicine, University of Milan, Milan, Italy).

19-22 October, **7th Canadian Symposium on Catalysis**, Edmonton (F. F. Gadallah, Alberta Research Council, 11315 -87th Ave, Edmonton, Alberta, Canada T6G 2C2).

20-23 October, **Etiology and Pathogenesis of Diabetes**, Toronto (Dr J. M. Martin, Research Institute, The Hospital for Sick Children, 555 University Ave, Toronto, Ontario, Canada M5G 1X8).

20-24 October, **International Conference on Current Nuclear Power Plant Safety Issues**, Stockholm (IAEA, Wagramstrassr 5 PO Box 100, A-1400 Vienna, Austria).

22-24 October, **HPLC Beginners Course**, Cheshire (HPLC Technology Ltd, PO Box 25, Wilmslow, Cheshire, UK).

22-24 October, **Workshop on TCDD and Related Compounds**, Rome (Prof. Dr O. Hutzinger, Laboratory of Environmental and Toxicological Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam, The Netherlands).

21-24 October, **European Offshore Petroleum Conference and Exhibition**, London (Society for Underwater Technology, 1 Birdcage Walk, London SW1, UK).

25 October, **The Analysis of the Insect Visual System**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

19-23 October, **Coal Conversion and the Environment**, Washington (Patricia M. Bresina, Biology Dept, Battelle, Pacific Northwest Laboratories, Richland, Washington 99352).

27-29 October, **Fundamental Mechanisms in Human Cancer Immunology**, Galveston (Margie Taylor, The University of Texas Medical Branch, 111-A Basic Science Building, Galveston, Texas 77550).

29-31 October, **New Trends in Antibiotics**, Milan (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).

30 October-1 November, **New Trends in Arterial Hypertension. Cellular Pharmacology and Physiopathology**, Deauville (Dr M. Worcel, Centre de Recherche, Roussel Uclaf, BP 9 93230, Romainville, France).

3-5 November, **Accelerators in Research and Industry**, Denton (J. L. Duggan, Physics Dept, North Texas State

University, Denton, Texas 76203).

4-7 November, **Impact of Climate on Planning and Building**, Israel (Dr A. Bitan, International Symposium on the Impact of Climate on Planning and Building, PO Box 16271, Tel Aviv, Israel).

5-6 November, **Thermal Storage of Solar Energy**, Amsterdam (Technisch Physische Dienst TNO?TH, PO Box 155, 2600 AD Delft, The Netherlands).

10-11 November, **Gas Monitoring for the 80s**, London (Carole Meads, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

11-13 November, **Underwater Mining Institute**, Georgia (Barbara J. Arnold, University of Wisconsin, Sea Grant Advisory Services, 1815 University Ave, Madison, Wisconsin 53706).

11-15 November, **Clinical Chemistry**, Milan (1st African and Mediterranean Congress of Clinical Chemistry, via Keplero 10, 20124 Milan, Italy).

11-14 November, **Magnetism and Magnetic Materials**, Dallas (I. S. Jacobs, General Electric CR&D, PO Box 8, Schenectady, New York 12301).

18-21 November, **Space Geodesy and its Applications**, Cannes (CNES, Dept des Affaires Universitaires, 18 Ave Edouard-Belin, 31055 Toulouse Cedex, France).

25-27 November, **Electro-Analytical Techniques**, London (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

26 November, **Research in a Declining Industrial Environment**, London (Administrative Secretary, The Research and Development Society, 47 Belgrave Square, London SW1, UK).

1-3 December, **Prostaglandins and the Cardiovascular System**, Antwerp (Ms L. Van den Eynde, Dept of Pharmacology, University of Antwerp, Universitaitsplein 1, B-2610 Wilrijk, Belgium).

1-4 December, **Atomic and Nuclear Methods in Fossil Energy Research**, Puerto Rico (Dr R. Filby, Nuclear Radiation Center, Washington State University, Pullman, Washington 99163).

1-12 December, **7th UICC Training Course in Cancer Research**, Melbourne (Dr A. W. Burgess, UICC Course, The Walter and Eliza Hall Institute, Royal Melbourne Hospital PO, 3050, Victoria, Australia).

8-11 December, **Lipid Metabolism and its Pathology**, Lisbon (Prof. Manuel Judice Halpern, Departamento de Bioquímica, Faculdade de Ciências Médicas da UNL, Campo dos Martíres da Patria, 1100 Lisbon, Portugal).

15-17 December, **Special Ceramics**, London (P. Popper, The British Ceramic Research Association, Queens Rd, Penkhull, Stoke on Trent, UK).

16-17 December, **Chromatography, Equilibria and Kinetics**, Brighton (Mrs Y. A. Fish, The Chemical Society, Burlington House, London W1, UK).

25-26 March, **Official Methods of Analysis and Stability Testing of New Drugs**, Cambridge (Dr D. Simpson, Analysis for Industry, Factories 2/3, Bosworth House, High St, Thorpe-le-Soken, Essex, UK).